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The Tohoku Journal of Experimental Medicine, Vol. 70, No. 4, 1959

Clinical Studies on Liver Catalase Activity in Gastric Carcinoma Cases

II. Experimental Supplement

By

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(Received for publication, May 25, 1959)

Following the clinical observations reported in the first paper, the aqueous extract of gastric carcinoma tissue was tested for the inhibitory effect on the catalase activity of the mouse liver, being studied the relation of this effect to the nature of the tumor as well as to the liver catalase activity of the patient.

Experimental

Method

1) Objects

Tumor tissues of 39 gastric carcinoma cases, in which subtotal resection of the stomach was performed, were used. They included some of the 58 cases examined in the first report.

2) Method

The aqueous extract prepared from the tissue was injected intraperitoneally into the mouse and the liver catalase activity was measured after 24 hours.

Injection material. A portion of the carcinoma tissue was dissected from the resected stomach, washed to remove the blood, mucus and necrotic matter, sliced, and then dried in a desiccator and powdered finely. The tissue powder so prepared was extracted with ten times as much distilled water and the extract separated by centrifugation was condensed under reduced pressure at a low temperature, and then dried.

Before using, the dried extract was dissolved into sterile physiological saline at 10 mg/cc.

Preparation of enzyme solution. The animals used were male mice of DD strain, each of which weighed about 15 g. They were fed with a definite feed composed of wheat, barley, bean cake and fish-meal.

Injection was performed intraperitoneally with a dosage of 10 mg. (1.0 cc. as a solution) per 10 g. body weight, referring to the previous

communication[1].

Twenty four hours after the injection, the mouse was killed by decapitation and laparotomized immediately. A piece of the liver weighing about 0.1 g. was taken from the ventral margin of this organ, washed with saline to remove the blood completely, then wiped and weighed. This was then masticated in a mortar with a small amount of quartz sand, to which was added pH 7.0 phosphate buffer solution to make a 1:1000 suspension, supernatant of the latter being ready for measurement.

Method for measurement. See the first paper. Three mice were used for one material and the data obtained were averaged.

Results

1) Liver Catalase Activities in Mice Injected with Aqueous Extract of Gastric Carcinoma Tissue

In Fig. 1 is shown the frequency distribution of the liver catalase activities in mice injected with 39 aqueous extracts of gastric carcinoma tissue, and of 22 control mice which were injected with plain physiological saline at a dose of 1.0 cc. per 10 g. body weight for comparison. The activities were 23.3 to 60.7, 44.1 ± 2.5 on an average[2], in the injected mice, being apparently lower than those in the control mice (41.7 to 83.2, 64.4 ± 4.7).

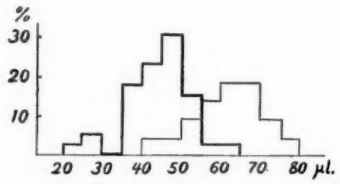

Fig. 1. Frequency distribution of liver catalase activities of mice when the tumor extracts were injected (heavy lines). Light lines show that of the control mice.

2) Relation of Catalase Lowering Activity to Morphological Feature of Tumor

See Table I. No significant difference was found in the liver catalase activity between the mice injected with the extracts prepared from the tumors of Borrmann's types I or II (three, and 24 cases, respectively) and those with the extracts of type III (12 cases), nor between the mice injected with the extracts of carcinoma adenomatosum (17 cases) and those with the extracts of carcinoma solidum (22 cases).

3) Relation between Liver Catalase Activity of Gastric Carcinoma Cases and Catalase Lowering Activity of Tumor Extracts

TABLE I

Liver Catalase Activity of Injected Mice According to Types of Tumor from which the Injection Material was Extracted

Types of tumor		Cases	Liver catalase activity		
			Minimum	Maximum	Mean
Gross appearance	Types I & II	27	23.3	56.0	43.6±3.3
	Type III	12	35.0	60.7	45.3±4.3
Histological feature	Adenomatosum	17	36.3	54.8	45.1±2.9
	Solidum	22	23.3	60.7	43.3±4.1

In Fig. 2, the ordinate shows the liver catalase activity of the patient with gastric carcinoma and the abscissa the liver catalase activity of the mouse injected with an aqueous extract prepared from the tumor of this patient. No significant correlation was found between the two values.

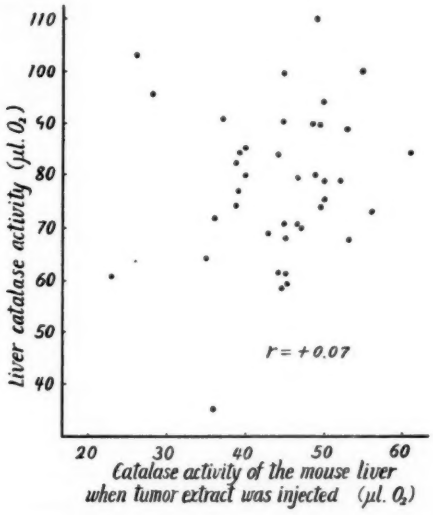

Fig. 2. Diagram showing the relation between the liver catalase activity of a mouse injected with the tumor extract and that of the host of the same tumor. The correlation was insignificant stochastically.

DISCUSSION

Greenstein *et al.*[3] observed that the lowered catalase activity of the rat liver induced by subcutaneous implantation of hepatoma 31 was restored when the tumor was extirpated, and were led to the conclusion

that the lowering of the liver catalase activity in tumor-bearing animals is a far-reaching effect elicited from the existence of the tumor. And, failing to produce the lowering of the catalase activity by intraperitoneal injection of the tumor homogenate, they were of the opinion, at that time, that the living tumor is needed for such a phenomenon.[4]

Later, Nakahara and Fukuoka[5] found that a substance separated from the tissue of the human carcinoma lowers the catalase activity of the mouse liver when injected intraperitoneally, and this was confirmed by Greenfield *et al.*[6] in various tumors and in different animals.

As a part of a series of our studies on toxic substances contained in the cancerous tissue,[1][7][8][9] we have also reported previously that the aqueous extract of gastric carcinoma tissue inhibits the liver catalase activity of mice in vivo, and that a nucleoprotein fraction of the tissue constituent is the substance responsible for this effect.[1]

In the first report of the present work, it was revealed that the catalase activity in the liver is lowered in gastric carcinoma cases parallel to the extent of spread of the tumor, but with little dependence on the morphological features of the tumor. If so, how does the catalase lowering activity of the tumor extract vary according to the types of the tumor from which it was prepared? Is there any relation between this activity and the liver catalase level of a patient with the tumor? These are the subjects to be discussed here.

As shown in Table I, however, no close relation was found between the catalase lowering activity on one hand, and the macroscopic as well as microscopic types of the tumor on the other hand. This suggests that the catalase depressing substances are contained, at least in human gastric carcinoma, equally in various types of the tumor, and corresponds to the fact proved clinically in the first paper that the liver catalase activity had little relation to such morphological types of the tumor.

Greenstein and Andervont[10] reported that the liver catalase activities of tumor-bearing animals had close relation with the kind of tumors implanted to, or originated in, them. Though the results of the present work, clinical as well as experimental, seem to be incompatible with the report mentioned above, it can be understood that such varieties as various types in gastric carcinoma differ essentially from those among the experimental tumors produced from the different matrices by different procedures, and that the variety in the liver catalase activity of gastric carcinoma cases was due little to the difference in morphological types of the tumor, but greatly to the difference in growing or invasive energy of the tumor which is actuallized as the peritoneal dissemination and the lymph-nodal involvement.

Moreover, no distinct correlation was obtained between the catalase

lowering activity of the tumor extract and the catalase activity of the liver of the patient with the tumor from which the extract was prepared.

But, it is very important to notice here that the " catalase-lowering activity " in this work is that of a unit weight of the extract, and may be that of a unit weight of the tumor tissue even supposing the existence of an almost equal content of water-extractible substances in tumor tissues. Therefore, when we make a resiprocal of the liver catalase activity of the mouse (in $\mu l.$ of oxygen) injected with the extract present the catalase depressing activity per unit weight of a tumor and use a square measure of a tumor (a product of the longitudinal by transverse diameters, in $cm.^2$) in place of the weight of the tumor, the product of these two values may be an index showing more precisely the catalase lowering activity of a tumor in vivo, and hereafter in this paper, it will be called the " presumed catalase-depressing activity " of the tumor.

TABLE II

Liver Catalase Activity of Gastric Carcinoma Cases According to " Presumed Catalase-depressing Activity " of Tumors

Presumed catalase-depressing activity	Cases	Liver catalase activity		
		Minimum	Maximum	Mean
Class I	10	67.9	100.3	84.6± 7.6
II	10	59.4	109.7	79.6±13.4
III	10	60.6	94.2	76.6± 6.8
IV	9	35.3	92.7	70.5±12.8

Such presumed activities of 39 tumors examined were 0.04 to 2.64, showing a wide range. Of course the numerical values themselves are meaningless. When we group the examined tumors into four groups according to their presumed catalase-depressing activities, each of them containing the same number of cases, the less liver catalase activities were found in the group including cases with tumors of the greater catalase-depressing activity (Table II). An inverse correlation was thus demonstrated between the liver catalase activity of cancer cases and the catalase-depressing activity of the tumor.

As to the patho-physiological mechanism of the catalase inhibition in tumor-bearing animals, there are also opinions that the product of the tumor does not affect the catalase activity, but that the synthesis of catalase is disturbed for lack of its constituents due to the rapid growth of the tumor.[11] But, the catalase lowering activity of the tumor components is a matter approved in general, and that the same process should be carries actually in tumor-bearing animals was demonstrated also by a parabiotic

experiment by Lucké *et al.*[12]

Close correlation of the two values, the liver catalase activity of cancer cases and the catalase-depressing activity of the tumor which was calculated, taking the tumor size into consideration, from the liver catalase activity of the mouse injected with the tumor extract, is thought to be a fact supporting the humoral mechanism in general patho-physiological effects of the malignant tumor.

Summary

Aqueous extracts were prepared from 39 gastric carcinoma tissues and injected into mice intraperitoneally, the liver catalase activity of which was measured. The results obtained are summarized as follows.

1. Liver catalase activities in mice injected with the tumor extracts were significantly lower than those in the control mice.

2. Catalase-lowering activity of a tumor extract was not dependent upon the macroscopic or microscopic features of the tumor.

3. Decrease in liver catalase activity of gastric carcinoma cases had little relation to the catalase lowering activity of the tumor extract, but showed close parallelism with the catalase-depressing ability of the tumor itself calculated under some assumptions.

References and note

1) Oh-Uti, Sato, Abe & Yamaguchi, Tohoku J. Exper. Med., 1957, **67**, 97.

2) In the text, as well as in the tables, arithmetical means were presented with 95 per cent confidence limits.

3) Greenstein, Jenrette & White, J. Nat. Cancer Inst., 1941, **2**, 283.

4) Greenstein, *ibid.*, 1943, **3**, 397.

5) Nakahara & Fukuoka, Jap. Med. J., 1948, **1**, 271.

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7) Yamaguchi & Oh-Uti, Tohoku J. Exper. Med., 1957, **67**, 73.

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10) Greenstein & Andervont, J. Nat. Cancer Inst., 1942, **2**, 345.

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The Tohoku Journal of Experimental Medicine, Vol. 70, No. 4, 1959

The Electron-microscopic Observation on the Pulmonary Alveolar Phagocytes of the Mouse*

By

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(Received for publication, May 27, 1959)

The present authors are studying the mechanism of invasion of inhaled dust into the pulmonary stroma, in relation with the problem of the pathogenesis of pneumoconiosis. In the present report, the authors will focus their attention on the electron-microscopic features of pulmonary alveolar phagocytes. Causing mice to inhale soot dust we examined their lung tissues under an electron-microscope, and payed special attention to the morphology of the cells covering the alveolar wall, especially, to the relation between the soot-ingesting cells and the other lining cells.

Experimental

Materials and Method

Normal adult mice were made to inhale soot produced by burning xylene, for one hour and sacrificed one to 48 hours thereafter. Some others were subjected to the inhalation 30 min. each per day for one week and sacrificed on the last day. Untreated mice were used as controls.

Small pieces of the lung tissue of mice were immersed into Palade's[1] Fixative, one per cent osmium tetroxide solution buffered at about pH 7.2 with veronal-acetate buffer. Fixation was for 30–40 minutes, after which the specimens was washed in distilled water for a short time, dehydrated in a series of ethanol and embedded in methacrylate. HU-10 type electron microscope was used for observation of ultra-thin section. Remained lung tissue was used for formalin fixation and light-microscopic observation.

* Read at the 15th Annual Meeting of the Society of Electron Microscopy held in Sendai on 15 May, 1959.

Results

In the lungs of mice made to inhale soot, the following sorts of cells were found lining the wall of the alveoli:

I. Small-sized smooth-contoured cells with a nucleus taking up a rather large part of the cell body, and containing a few of elliptic or more elongated rather small mitochondria. The endoplasmic reticulum (E.R.) is not well developed, some small saccular or tubular shreds of it being found sporadically. The peripheral parts of the cell bodies pass over into thin cytoplasmic sheets, which are homogenous and structureless and form a triple layer with the thin part of the capillary endothelium and the somewhat clearer basement membrane between them. In this thin cytoplasmic sheet no mitochondria can be found, but some saccular or dropletlike small structures are seen.

II. Cells with cytoplasm rich in organelles, with microvilli forming on a part of their cell membrane, sometimes giving the appearance of the cell being in contact with other cells through them, or sometimes in direct contact with the basement membrane. Here, the particularly conspicuous finding is the electron-dense, lamellar or vortical corpuscles seemingly specific in existence to the lung. These have a definite limiting membrane, and sometimes, a part only of them is found in a vacuole. These cells contain mostly large-sized and round mitochondria, the cristae are irregularly and loosely arranged and it is characteristic of their ground substance that it is relatively electron-dense and homogenous. Some of these simulate " microbody " described by Rhodin.

Of the above lamellar structures, some had their structure comparatively well preserved, but some vacuoles which contained them were fused with the neighboring vacuoles into large vacuoles and some again were broken up, in extreme cases above mentioned lamellar bodies being scattered out of the cell body in the alveolar space. The last-mentioned phenomenon may be due to artefacts, but since no such pictures could be seen in the cases of vacuoles containing soot dust or mitochondria, this finding seems at least to indicate the physicochemical properties, especially, the visco-elasticity, of the vacuolar wall. Among these cells, soot was found taken in an very rare occasions only, and even in these rare cases, the quantity of ingested soot was very low.

Such I and II cells were found in the untreated control mice as well.

III. Phagocytes containing ingested soot. These have rich cytoplasm of relatively low electron density, and the nucleus is rather small in proportion to and stands eccentric in the body. The cell membrane has fine irregular infoldings and processes which are distinguishable at a glance from the microvilli of the II cells above. The mitochondria are devoid of

the characteristics of the II cells above, but are mostly common, elongated in form, with definite and regularly aligned cristae.

The ingested soot particles were 0.05–0.1 μ in diameter, 20–50 of them being aggregated into groups of 1–2 μ across, which were found scattered about in the cytoplasm. These groups of soot particles were sometimes in vacuoles with clear, nearly vacant background and often marked off by a limiting membrane, but particles were sometimes in positions as if extruded into cytoplasm from such a vacuole.

Pictures of soot-ingesting cells closely adhering to the stromal connective tissue or the basement membrane connected thereto through irregularities on their surface were also obtained. The phagocytes with soot taken up in a quantity usually lack in the lamellar structure to be called the characteristic structure in II cells above, but it is true that in some exceptional cases soot phagocytes also show this characteristic formation.

Comment and Conclusion

If we take the phagocytosis of soot particles, the presence of electron dense lamellar structure seemingly specific to pulmonary cells, the nature of the mitochondria contained and the existence of microvilli as the principal criteria, the cells on the surface of the alveoli may be classified into 3 major groups. I and II are seemingly identical with the types described by Yasuda[2] as Type A and Type B epithelial cells. But here, our attention was drawn to the fact that there were cells that clearly showed the characteristics of II cells and yet had ingested a small quantity of soot and those that had taken in a large quantity of soot and yet were not devoid of all the characteristics of the II cells. Such transition-type cells appeared more prominently in the mice subjected to soot inhalation for a week. It is difficult whether the cells of II and III are originally of the same type or originally and functionally different cells here show apparent transitional findings due to changes in the environment.

It is also difficult to decide at present whether the dense lamellar structure found in our II cells or Yasuda's Type B epithelial cells (found also in the untreated control mice) is produced by a transformation of the mitochondria (Schulz[3]) or otherwise. It seems more reasonable to consider the vacuoles of phagocytes as related with the E.R. system as are the digestive vacuoles in general, than as related with the mitochondria. The extrusion of carbon particles through the vacuolar wall shows that the wall is damaged here and lets the particles into the cytoplasm. Then, obviously the metabolism in the cell is highly disturbed. Phagocytes often lose their connection with the neighboring cells, the stromal connective tissue or the basement membrane and desquamate into the alveolar

space. But since we found nothing suggestive of a directional (stromatotropic) intracellular transport, we cannot agree with the opinion of Gieseking[4]. We are inclined to regard phagocytic action of pulmonary alveolar phagocytes against inhaled foreign particles as defensive mechanism.

References

1) Palade, J. Exper. Med., 1952, **95**, 285.
2) Yasuda, Acta Path. Jap., 1958, **8**, 189.
3) Schulz, Beitr. path. Anat., 1958, **119**, 71.
4) Gieseking, Erg. allg. Path., 1958, **38**, 92.

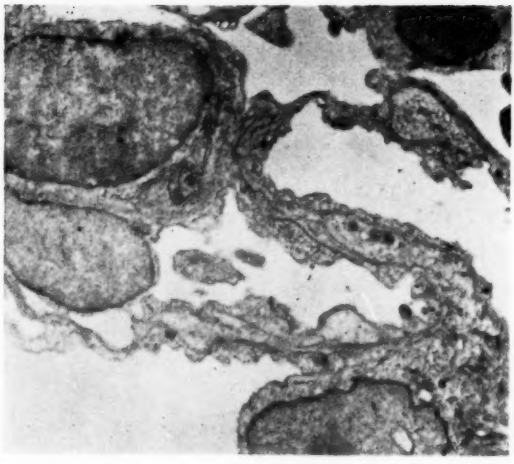

Fig. 1. I cells in the upper-left and the lower-right parts. Note their smooth contour, comparatively large nucleus and scanty mitochondria. ×7,800

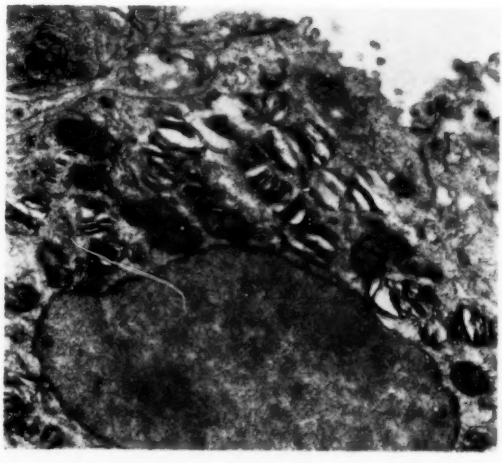

Fig. 2. II cells. The cytoplasm filled up with lamellar osmiophilic corpuscles and large-sized mitochondria. Microvilli formation is seen. ×14,000

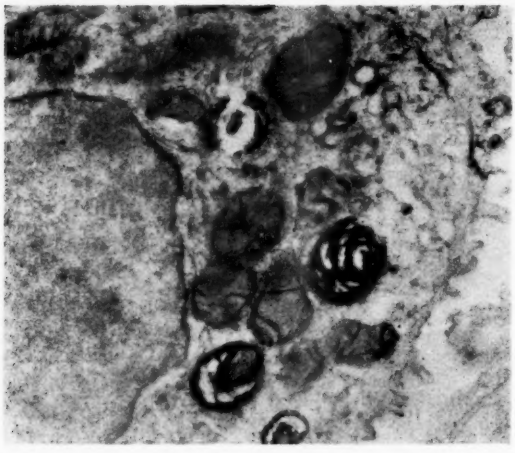

Fig. 3. Ditto. Lamellar corpuscles in various shape. The mitochondria are round or elliptical, the ground substance is considerably electron-dense and the cristae are irregularly arranged. ×22,000

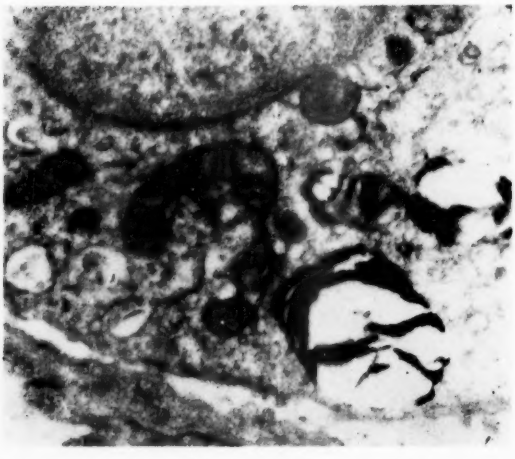

Fig. 4. The vacuole containing a lamellar corpuscle is enlarged. Sometimes such a vacuole is obviously unified with the neighboring vacuole. ×22,000

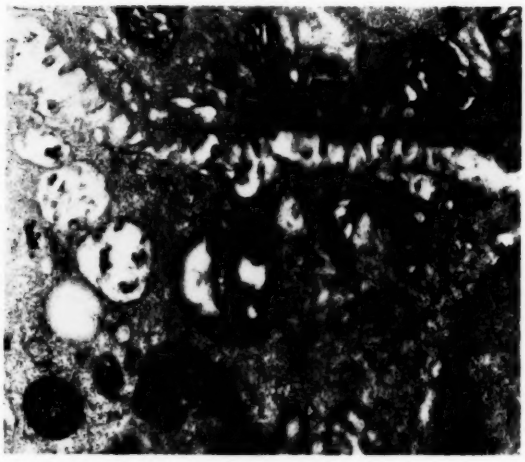

Fig. 5. Cells presumably of the II found in a control mouse. Two cells in mutual contact through microvilli and both containing multiform osmiophilic corpuscles. ×17,500

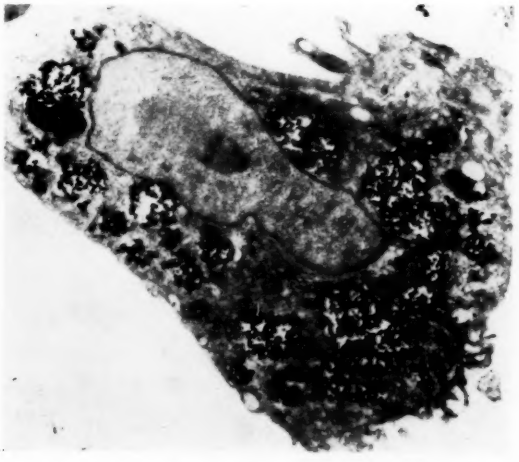

Fig. 6. Phagocytes. Fine soot particles in groups lying scattered in the cytoplasm. A few of small elongated mitochondria are seen. ×11,000

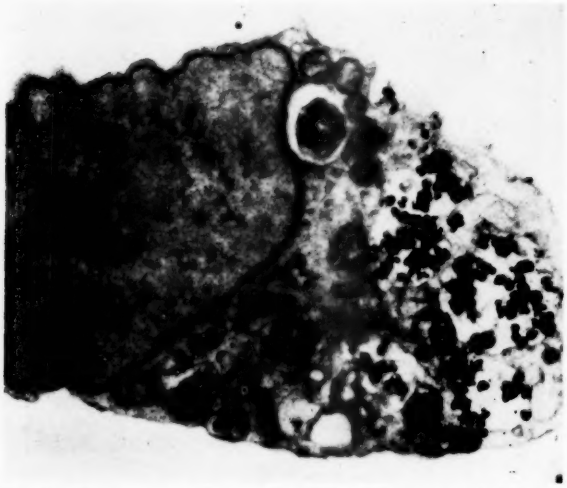

Fig. 7. Phagocytes in the mouse lung subjected to soot inhalation for one week. Note large sized mitochondria and a few somewhat lamellar corpuscles. ×18,500

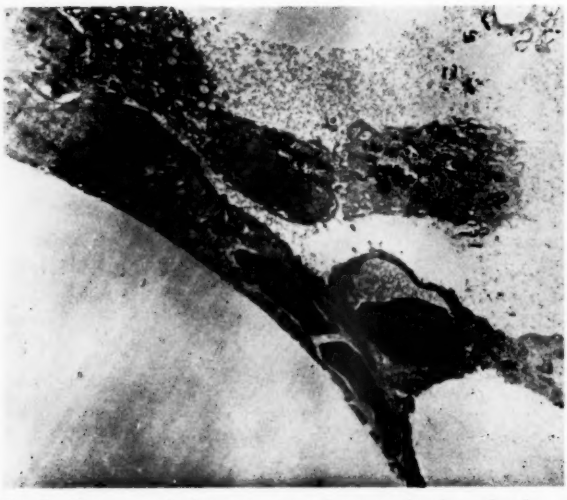

Fig. 8. Degenerated phagocytes now desquamating into alveolar space. ×4,100

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Descending Spinal Pathway Subserving Adrenaline Secretion of the Adrenal Gland in the Toad

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(Received for publication, June 9, 1959)

In my previous study it was found that in toads the section of the splanchnic nerves prevented the depletion of adrenaline in the adrenal gland producible by insulin hypoglycemia.[1] This indicates that insulin hypoglycemia acts on the adrenal medulla through the central mechanism rather than directly.

It has been well established that in dogs the augmentation of adrenaline secretion causable by insulin hypoglycemia is abolished by the transection of the spinal cord at the level of the lowest cervical segment.[2][3] In the present study it was found at first that the drecrease in the adrenaline content of toad adrenal gland causable by insulin hypoglycemia was prevented by the spinal cord transection at the level of the second spinal roots. Thus insulin hypoglycemia was proved to deplete adrenaline of toad adrenal gland in acting on the adrenaline-secretory center situated above the level of transection.

Then, in order to know whether the descending spinal pathway of the adrenaline-secretory fibers decussates or runs uncrossed, insulin experiments were performed in toads, in which the hemisection of spinal cord was made.

Experimental

Methods

Toads of either sex were used. The transection or hemisection of the spinal cord was made at the level of the second spinal roots after opening the dura mater. Experiments were performed about 4 days after the spinal cord operation.

Insulin was given subcutaneously in a dose of 15 units per kg. of body weight. Ten hours after insulin injection the animals were killed and

the adrenal glands were immediately taken out together with kidneys on both sides. Renal tissue was cut off from the adrenal gland as completely as possible. Adrenal gland was then extracted with 4% trichloracetic acid. Control experiments were performed without injection of insulin.

Adrenaline and noradrenaline contents of adrenal extracts were estimated by using the permanganate method of Suzuki and Ozaki.[4] As the reference standards L-adrenaline (Merck) and DL-noradrenaline (Sankyo) were used.

Results

(1) Experiments in toads subjected to spinal cord transection

In 7 toads, weighing from 200 g. to 380 g., control experiments were made without injection of insulin. In 9 toads, weighing from 200 g. to 390 g., subcutaneous injection of insulin was done. The results are summarized in Table I.

TABLE I

Effect of Insulin on the Adrenaline and Noradrenaline Contents of the Adrenal Gland in Toads, in Which the Transection of the Spinal Cord Was Made at the Level of the Seond Spinal Roots

	Left or right	Adrenaline (mg.) Mean±standard error of the mean (Minimum-maximum)		Noradrenaline (mg.) Mean±standard error of the mean (Minimum-maximum)	
		per gland	per kg. of body weight	per gland	per kg. of body weight
Control (7 toads)	L.	0.052±0.012 (0.037-0.072)	0.197±0.016 (0.177-0.225)	0.034±0.007 (0.028-0.043)	0.132±0.012 (0.113-0.150)
	R.	0.052±0.013 (0.037-0.068)	0.197±0.023 (0.168-0.243)	0.032±0.006 (0.024-0.041)	0.122±0.008 (0.108-0.132)
Insulin injected (9 toads)	L.	0.053±0.011 (0.040-0.069)	0.180±0.023 (0.143-0.210)	0.035±0.006 (0.025-0.043)	0.121±0.017 (0.090-0.150)
	R.	0.054±0.012 (0.040-0.071)	0.186±0.018 (0.154-0.209)	0.035±0.006 (0.026-0.043)	0.123±0.016 (0.095-0.145)

In control experiments the mean adrenaline content of the adrenal gland was 0.052 mg. per gland and 0.197 mg. per kg. of body weight in the left as well as in the right gland. The mean noradrenaline content was 0.034 mg. and 0.032 mg. per gland in the left and the right gland, respectively. Computed per kg. of body weight, it was 0.132 mg. and 0.122 mg., respectively.

In insulin experiments, the adrenaline content was on the average 0.053 mg. and 0.054 mg. per gland in the left and the right gland, re-

spectively. It was 0.180 mg. and 0.186 mg. per kg. of body weight, respectively. The mean noradrenaline content was estimated as 0.035 mg. per gland in the left and the right gland, as well. It was 0.121 mg. and 0.123 mg. per kg. of body weight in the left and the right adrenal gland, respectively.

In comparison of the adrenaline content of the adrenal gland in insulin injected toads with that in control ones, no statistically significant difference was found ($P>0.10$).

(2) *Experiments in toads subjected to the hemisection of the spinal cord*

The hemisection of the spinal cord was performed on the right side in 14 toads, weighing from 250 g. to 430 g. Six of them were used in control experiments and 8 others in insulin experiments. The results are shown in Table II.

TABLE II

Effect of Insulin on the Adrenaline and Noradrenaline Contents of the Adrenal Gland in Toads, in Which the Hemisection of the Spinal Cord Was Made on the Right Side at the Level of the Second Spinal Roots

	Left or right	Adrenaline (mg.) Mean±standard error of the mean (Minimum-maximum)		Noradrenaline (mg.) Mean±standard error of the mean (Minimum-maximum)	
		per gland	per kg. of body weight	per gland	per kg. of body weight
Control (6 toads)	L.	0.066±0.012 (0.047-0.083)	0.210±0.022 (0.179-0.240)	0.040±0.005 (0.030-0.045)	0.129±0.017 (0.100-0.144)
	R.	0.065±0.012 (0.047-0.086)	0.209±0.020 (0.179-0.230)	0.040±0.005 (0.028-0.048)	0.128±0.016 (0.106-0.155)
Insulin injected (8 toads)	L.	0.049±0.007 (0.038-0.058)	0.160±0.014 (0.144-0.178)	0.040±0.006 (0.031-0.052)	0.135±0.009 (0.118-0.148)
	R.	0.072±0.010 (0.053-0.089)	0.233±0.014 (0.203-0.251)	0.044±0.007 (0.032-0.048)	0.142±0.012 (0.125-0.160)

In control experiments, the adrenaline content per gland was on the average 0.066 mg. in the left and 0.065 mg. in the right gland, respectively. Computed per kg. of body weight, it was 0.210 mg. and 0.209 mg. in the left and the right gland, respectively. The mean noradrenaline content was 0.040 mg. per gland in the left and the right gland, as well. It was 0.129 mg. and 0.128 mg. per kg. of body weight in the left and the right gland, respectively.

In insulin experiments, the adrenaline content per gland was estimated on the average as 0.049 mg. in the left and 0.072 mg. in the right gland, respectively. Computed per kg. of body weight, it was 0.160 mg. in the

left and 0.233 mg. in the right gland, respectively. The mean noradrenaline content was 0.040 mg. and 0.044 mg. per gland in the left and the right gland, respectively. It was 0.135 mg. and 0.142 mg. per kg. of body weight in the left and the right gland, respectively.

In comparison of the adrenaline content of the right gland in insulin treated toads with that in control ones, there is no significant difference ($P>0.10$). However, comparing the adrenaline content of the left adrenal gland in insulin injected toads with that in control ones, the former is definitely smaller than the latter. The difference is statistically highly significant ($P<0.001$).

Discussion

In the first series of experiments it was proved that the decrease in the adrenaline content of the adrenal gland in toads causable by insulin hypoglycemia was nullified by the transection of the spinal cord at the level of the second spinal roots. In the second series of experiments it was demonstrated that in toads the hemisection of the spinal cord abolished the depletion of adrenaline causable by insulin hypoglycemia in the ipsilateral gland but not in the contralateral gland. Thus it is reasonable to conclude that in the toad the descending spinal pathway of the adrenaline-secretory fibers to the adrenal gland runs without crossing below the level of the second spinal roots.

In the dog it has been proved by Suzuki *et al.*[3] that the descending spinal pathway subserving the adrenaline secretion of the adrenal gland decussates partially in the spinal cord. It will be probable that the descending spinal pathway is not necessarily the same in different animal species.

Summary

In toads the transection or hemisection of the spinal cord was made at the level of the second spinal roots. After 4 days insulin was given. The adrenaline and noradrenaline contents of the adrenal gland were estimated by the permanganate method.

The decrease in the adrenaline content of the adrenal gland causable by insulin hypoglycemia was found to be prevented by the transection of the spinal cord. By the hemisection of the spinal cord it was also abolished in the ipsilateral gland but not in the contralateral gland.

Thus it is concluded that in the toad the descending spinal pathway subserving adrenaline secretion of the adrenal gland does not decussate.

I wish to express my gratitude to Prof. T. Suzuki for his kind advice.

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Experimental Studies on the Functional Condition of Reticuloendothelial System, Especially with Reference to the Various Manifestation Types of the Leprous and Tuberculous Foci

By

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Introduction

Long-continued studies in our department have revealed the fact that the functional condition of the reticuloendothelial system (RES) exerts significant influences upon the various manifestation types of the inflammatory foci. When its functional activity is promoted, the cellular proliferation and metamorphosis are rapidly and conspicuously provoked. On the contrary, its lowered functional activity results in the weakened cellular response corresponding to its stage and degree. It has been also revealed that the functional condition of the RES can be approximately shown by observing the cellular forms in the inflammatory foci.[1)-5),8)-11)]

The authors made the following experiments to elucidate the problem of how functional conditional differences of the RES changes the type of manifestation of such diseases, such as tuberculosis and leprosy which show the various manifestation types, based on the experimental records mentioned above.

Studies on the Leprous Foci of the Experimental Mice

(I) Experimental Materials and Methods

The authors adopted typhoid vaccine injection as a way of activating the functional condition of the RES. Marked effect of the vaccine on the function of RE cells was examined by Dr. Ohta of our department.[14)] Beforehand, a small quantity of the vaccine (0.2 cc.) had been injected in the peritoneum of mice (0.2 cc./20 g., one injection). Evans blue injection was used as another way of lowering the functional condition of

the RES (0.5%/0.1 cc./20 g., every other day, totalling five injections). Cardiolipin, one of the constituents of the rat leprosy bacillus, is closely connected with antigen-antibody reaction mainly in leprosy of the lepromatous type[13] and was injected as the third pretreatment (0.5 mg./0.2 cc./20 g., every other day, totalling 15 injections). According to Dr. Ohta,[14] cardiolipin generally inhibits the function of the RES.

Two days after the three kinds of pretreatment mentioned above were injected, each dd/T mouse was injected with living rat leprosy bacilli subcutaneously (10^{-2}/0.2 cc., Hawaiian and Kumamoto strains). Observation of manifestation findings in subcutaneous foci of leprous mice was continued until the thirteenth week from the cytohistopathological point of view, and a comparison was made with the control mice which have not received these pretreatments. Two to three mice were killed every week, and the sliced samples of their subcutaneous tissue, lymph nodes, liver, spleen, lung, kidney and genital organs were stained with H.E., PAS, PAP silver impregnation and Ziehl-Neelsen's stain. Furthermore, cytological observations by supravital staining with neutral red and Janus-green, May-Giemsa staining and under the phase contrast microscope were made.

(II) Records of the Experiments

Following are the results of lepramatous changes observed in both group of the control mice and in another group of mice which received pretreatments. Observation limited to the subcutaneous tissue of the mice, was carried on.

(1) Findings of the Group of Control Mice

Formation of rat leprosy tubercle was brought about by the monotonous proliferation of the so-called lepra cells. Lepra cells were filled with rat leprosy bacillus which kept their original shape.

In the foci of the leprosy rat, local histiocytes swell and become round. These histiocytes showed a tendency to become free. Formation of polynuclear histiocytes and that of neutral red rosette in their cytoplasm were hardly observable. Basophilism of cytoplasma meaning the proliferation of the cell that belongs to the RES was not so marked. The main findings of this case were that histiocytes were transformed into the large rounded cells.

(2) Findings of the Group of the Mice Injected with Typhoid Vaccine

Soon after the injection, proliferation of the spindle shaped cell and the polymorphous cell, medium in size, were seen in the foci of the leprous mice.

These cells took the form of the lepra cell as they came nearer and nearer the center of the foci. Furthermore, in the center of the foci, necrosis and degeneration were noticed. The foci showed centrifugal

aspects, and infiltration of the small rounded cell was conspicuous.

In the eleventh week, the lepra cells were hardly seen, and the foci demonstrated the so-called tuberculoid type composed of the epithelioid cells and the spindle shaped cells or the round mononucleated cells. In the same part, the presence of the polynucleated giant cells was observed dispersely (Fig. 1). Bacillus of rat leprosy is hardly seen and the remnants of them become droplets. In this case, local response of the histiocytes was caused mainly by the basophile cell and the numbers of these cells and their basophilism were noticeable. Tendensy for inversion of the fixed type histiocytes formed newly into lepra cells was weak (Fig. 2).

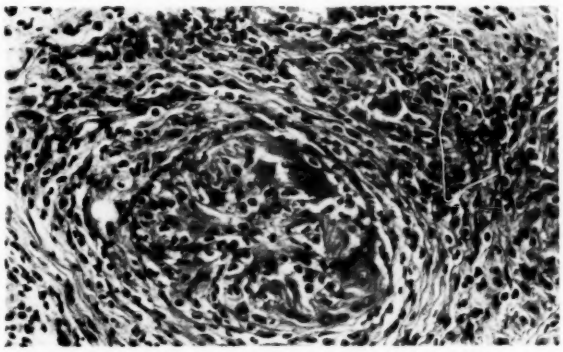

Fig. 1. Tuberculoid foci in the subcutaneous tissue of lepra mouse which received pretreatment of typhoid vaccine before 11 weeks.
Epithelioid cells and giant cells are seen (H.E.).

Fig. 2. The same case. (Supravital staining with neutral red and Janus -green).
Lepra bacilli are hardly demonstrable and remain in those phagocyted ruins as neutral red granules. (Phase contrast microscope).

(3) Findings of the Group of the Mice Injected with Evans blue

Local injuries of the tissue were remarkable from the beginning. Numbers of bacilli of rat leprosy were perceived in each cell body which appeared in the foci accompanying with the infiltration of leucocytes and diffuse necrosis or edema of the tissue. But these foci had a poor tendency towards formation of the lepra cell, and the medium-sized spindle-shaped rounded cells showed marked degenerative changes. Cytological observation made it apparent that in the cell body of the histiocytes shaped newly (their number was small), granules stained with Evans blue were accumulated. A number of vacuoles, large and small, was demonstrated. Nuclei of these cells atrophied (Fig. 3).

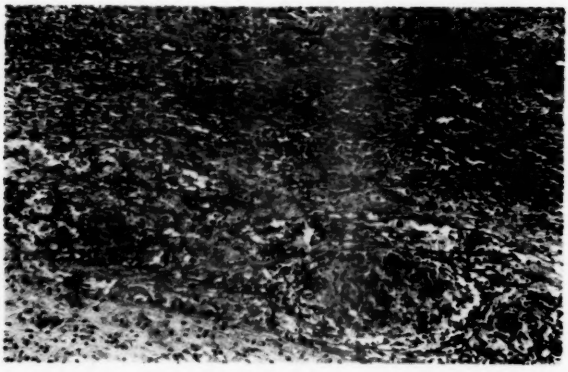

Fig. 3. Diffuse necrotic foci in the subcutaneous tissue of a lepra mouse which received pretreatment of Evans blue before 10 weeks (H.E.).

(4) Findings of the Group of Mice Injected with Cardiolipin

More typical and earlier formation of leprous foci similar to those of the control mice was noted (Fig. 4). These lepra cells contained vivid rat leprosy bacilli, and were poor in substance stainable with PAS. Tendency towards fibrous proliferation was very weak and capillaries were seen dispersed freely among these cell group. Basophilism of histiocytes was hardly recognizable, and their tendency for becoming free was not demonstrated (Fig. 5).

Studies on the Tuberculous Foci of the Experimental Mice

(I) Materials and Methods of the Experiments

(1) Animal Used: One hundred and ten healthy dd/N male mice. Their body weight was about 20 g., provided that the functional state of the reticuloendothelial system have had been ascertained to be within normal limits.

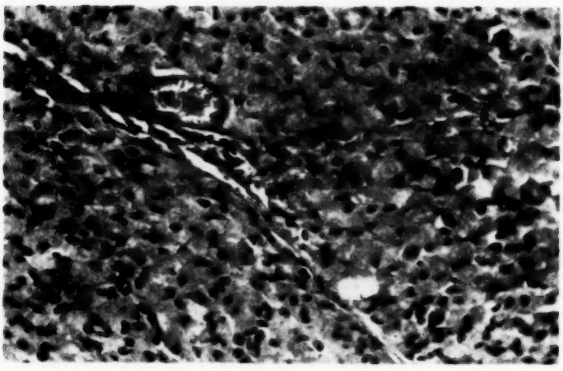

Fig. 4. Typical lepromatous foci in the subcutaneous tissue of a lepra mouse which received pretreatment of cardiolipin before 13 weeks. Uniform proliferation of lepra cells are seen (H.E.).

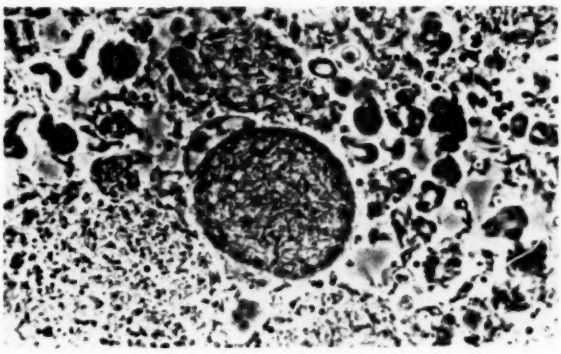

Fig. 5. The same case. (Supravital staining with neutral red and Janus -green).
Lepra cells are filled with lepra bacilli (Phase contrast microscope).

(2) Pretreatment: Same as those mentioned above.

(3) Methods of Experiments:

Two days after the first injection of pretreatment, $H_{37}Rv$ dried human type tubercle bacilli diluted with physiological saline (10^{-3mg}/0.1 cc.) were injected into the vein of tail of both groups of the mice.

Three to five mice were killed every week until the eighth week, and the sliced samples of their lung, liver, spleen, lymph nodes, intestinal tracts and kidney were stained with H.E., PAS, PAP silver impregnation and Ziehl-Neelsen's stain.

(II) Records of Experiments

Tuberculous change of both group were examined in the lung of both groups of mice.

(1) Findings of the Control Mice

Three weeks after the injection of tubercle bacillus stated above, the increase of cells in the alveolar wall was observed, and the formation of small tubercles composed of the middle sized rounded or oval shaped cell were found around small arteries. The foci characterized by the cellular proliferation increased their number gradually in subpleural part. The cells showed swelling and vacuole droplet degeneration.

Tuberculous foci, which appeared mainly around arteries during the sixth to the eighth week, were composed of the same kind of cells mentioned above. Appearance of the giant cell derived from these cells was not clear. Accompanying the vacuole degeneration of the epithelioid cell, leucocytic infiltration and exudative fluid were observed, but the formation of the caseous foci was not found. In the material stained with the Ziehl-Neelsen's method, the formation of the caseous foci was observed in bundle form parallel to the increase of the vacuole cell after the fourth week. The foci contained fairly large amounts of the droplet cell.

(2) Findings of the Group of the Mice Injected with Typhoid Vaccine

In contrast to the control group, marked formation of productive tubercle was noted. The boader of the tubercle after the fourth week was clear. Exudation and leucocytic infiltration were hardly seen. The kind of cells that constitute the foci were the medium-sized and the small rounded cell and epithelioid cell derived from them. Degeneration of those cells was not seen (Fig. 6). By means of PAS stain, PAS stain posi-

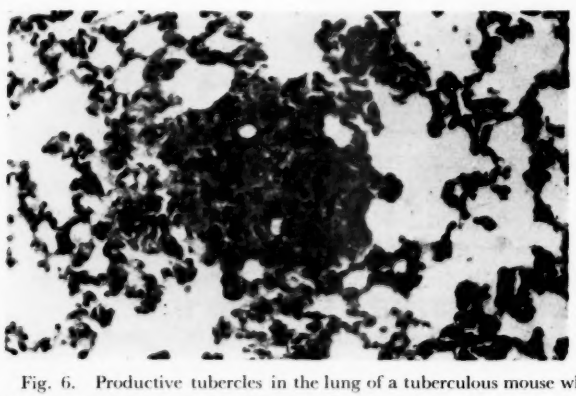

Fig. 6. Productive tubercles in the lung of a tuberculous mouse which received pretreatment of typhoid vaccine before 8 weeks.
Small and middle sized cells and epithelioid cells are seen in the tuberculous foci (H.E.).

tive granules were seen to be arranged in rosette shape. The bacillus was observed as droplets (Fig. 7). This meant that the functional state of the RES raised.

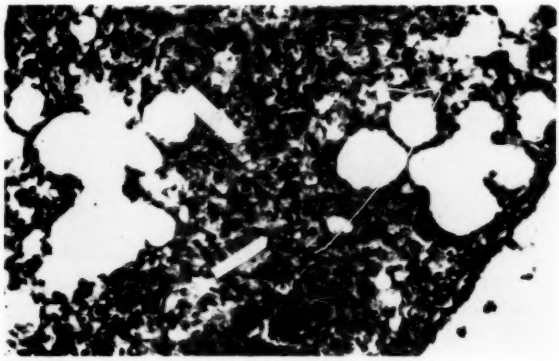

Fig. 7. The same case. (Ziehl-Neelsen's stain).
Tubercle bacilli are difficult to find in the foci.

(3) Findings of the Group of Mice Injected with Evans blue

Numbers of speckled tubercle composed of the medium-sized droplets cells appeared in the third week. Leucocytic infiltration and storage of exudate were fairly conspicuous. The fourth to the fifth week, the foci showed changes of diffuse caseous pneumonia. The sixth to the eighth week, degenerative changes of the confluent foci become conspicuous, and the central parts of it appeared to be in necrosis, and cavity formation was observed. Ziehl-Neelsen's stain made it clear that tubercle of the third week contained fairly amounts of the tubercle bacillus corresponding to the increased number of the vacuole cell. After the third week, the number and spread of the bacillus increased rapidly in the cavity mentioned above (Fig. 8). The presence of numbers of bacillus was confirmed, as if they had been cultured in a pure medium. But all through these processes cellular digestion of bacillus was very slight (Fig. 9).

(4) Findings of the Group of Mice Injected with Cardiolipin

Findings of this group were nearly the same as those of the control group. However, the formation of the productive foci was rather strong, that is to say, the foci showed a tendency to diffuse and spread ; the droplets degeneration of the medium sized cell appearing around the arteries was conspicuous. Marked exudation and leucocytic infiltration were observed (Fig. 10).

Summary and Consideration

As indicated in the above statements, the authors tried to clarify the

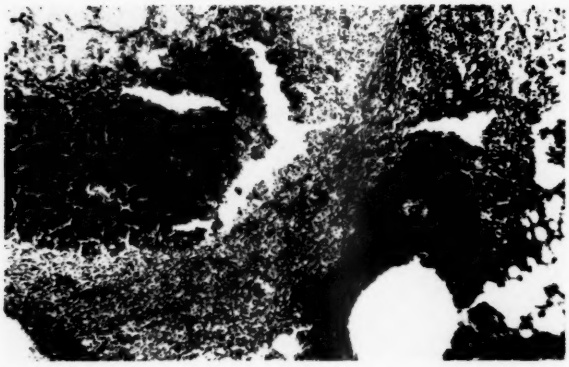

Fig. 8. Exudative foci in the lung of a tuberculous mouse which received pretreatment of Evans blue before 8 weeks.
Large tuberculous cavity is formed (H.E.).

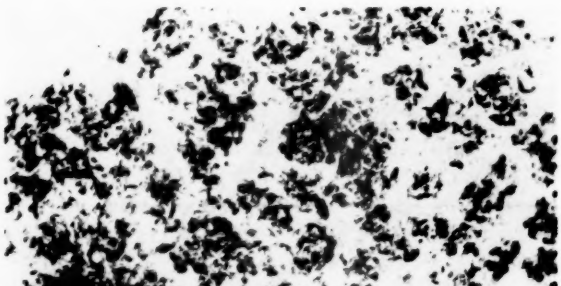

Fig. 9. The same case. (Ziehl-Neelsen's stain).
Tubercle bacilli are more numerously seen in this case than in the control.

role of the RES as the main factor of the living body that is concerned with the various histopathological metamorphosis and types of focus manifestation of such diseases such as leprosy and tuberculosis.[3)6)16)17)]

Generally speaking, basophilism of the RE cell has a relationship with new cellular production, and also it is considered to be the necessary pre-condition of phagocytosis of the RES.[1)-5)11)12)]

The RE cells show both proliferation and various histological metamorphosis, and this system responds to the functional demands of the living body.[7)-11)] For example, the appearance of the small rounded free cell is the reflection of the increased functional state of the RES, and on the contrary, weak appearance of these cells means the lowered functional

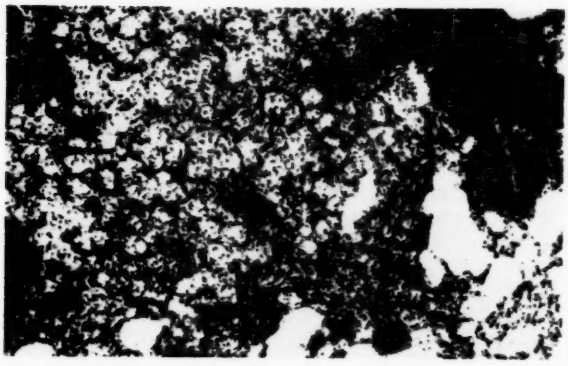

Fig. 10. Diffuse productive and exudative foci in the lung of a tuberculous mouse which received pretreatment with cardiolipin before 8 weeks.
Many middle sized and degenerated cells are seen in the tuberculous foci (H.E.).

state of the RE cells.

Furthermore, neutral red granules and PAS positive substance are supposed to be associated with the digestive mechanism of the RE cells for the foreign bodies.

In this study, the authors used this fact as a standard for determining the functional state of the RES from the morphological point of view. Based on the experimental results, analyses of the phase of the tuberculous and leprous foci of the group of mice whose RES was lowered or raised were made respectively. The leprous foci which were produced in the subcutaneous tissue of the mice by the injection of the lepra bacillus were typical lepromatous type in the control. In the group of mice which received the cardiolipin, earlier and more marked appearance of the foci of lepromatous type than that of the control. In the group injected with typhoid vaccine, the appearance of the tuberculoid foci was noted.

The tubercle foci formation in the lungs of the mice infected with human type tubercle bacillus is somewhat affected by the functional state of the RES. The same results can be stated about the foci of the rat leprosy. The tuberculous foci formation corresponding to the pretreatments, is characteristic in each case, and the kind and number of the cells taking part in the foci formation vary. These facts and the staining tendency of the tubercle bacillus within the foci presents enough material to say that the RES exerts influence upon the foci formation.

In the control mice which did not received pretreatment, proliferative foci formation was aroused. This type of the focus formation was accentuated by the increased functional state of the RES; under the lowered

functional state of the RES, formation of rather worse proliferative foci was demonstrated. Moreover under the disturbed condition of the RES, formation of exudative foci is characteristic. Summarizing the results mentioned above, it can be deduced that the difference of the functional condition of the RES in the living body exert very significant influences upon the various manifestation types of such disease as leprosy and tuberculosis. Therefore the authors believe that the function of the RES plays an important role as a non-specific resistance of the living body.[7)12)15)]

Conclusion

Results of the experiment on mice leprosy are as follows: 1) Promotion of the functional condition of the RES resulted mainly in the formation of tuberculoid typed foci. 2) Disturbance of the functional condition of the RES resulted in the formation of diffuse necrotic foci in the early weeks. 3) Inhibition of the functional condition of the RES resulted in the early formation of typical leprous foci with contrast to that of the controls.

Results of the experiment on mice tuberculosis are as follows: 1) Promotion of functional condition of the RES resulted in the formation of marked productive foci as compared with that of the controls. 2) Disturbance of the functional condition of the RES resulted mainly in the formation of the exudative foci. Caseous necrosis and cavity formation were also noticed. 3) Inhibition of the functional condition resulted in the formation of more degenerated foci in contrast to that of the controls.

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Effect of Hexamethonium on the Adrenaline Secretion in Response to Acetylcholine of the Denervated Adrenal Gland

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It is now fairly generally accepted that hexamethonium has the blocking effect on the sympathetic ganglion.

As regards its effect on the adrenal medulla, Schachter[1] reported that in dogs the hypoglycemic effect of insulin was intensified by the application of hexamethonium. According to him it is probably due to the blocking effect of the latter on the adrenal medulla. In estimating directly the adrenaline secretion rate of the adrenal gland, Yamashita found that in the dog the augmentation in the adrenaline secretion causable by acetylcholine[2] and by insulin hypoglycemia[3] was greatly diminished by hexamethonium. He concluded that hexamethonium paralyzed the adrenal medullary cells. The above investigations aimed to elucidate the effect of hexamethonium on the innervated adrenal medulla. Little is known, however, of its effect on the denervated gland.

Concerning the effect of hexamethonium on the denervated sympathetic ganglion, Perry and Reinert[4] demonstrated in cats that the blocking effect of hexamethonium on the ganglionic response to acetylcholine was abolished by the degenerative section of preganglionic nerve fibers. They used the retraction of the nictitating membrane as the indicator of ganglionic response.

The present study was undertaken to know whether the adrenaline-secretory effect of acetylcholine on the denervated adrenal medulla is suppressed or not by hexamethonium.

Experimental

Methods

Nine dogs, weighing from 6.6 to 13.5 kg., were used. The denervation of the adrenal gland was done by cutting the ipsilateral splanchnic nerves through the lumbar route. All the experiments were performed

under Evipan-sodium anesthesia. The adrenal venous blood was collected through a small cannula inserted into the adrenal vein just lateral to the gland, according to the method of Satake *et al.*[5] The adrenaline content of the adrenal venous blood specimens was estimated by the Bloor and Bullen's arseno-molybdic acid method,[6] adrenaline (Merck) being used as the reference standard. In most experiments the blood pressure was recorded by the mercury manometer connected with femoral artery. For the intravenous injection of chemicals the saphenous vein was prepared and a small cannula was inserted into the vein.

After atropinizing the animal, acetylcholine chloride (Hoffmann La Roche) was injected intravenously in a dose of 2 mg. per kg. of body weight in 15 seconds. After a while hexamethonium (Bistrium bromide, Squibb) was injected intravenously in a dose of 0.75 mg. per kg. Then, acetylcholine in the same dose as before was injected again. The adrenal venous blood was collected before injections of acetylcholine, as well as during the first and the second 60-second periods after the start of acetylcholine injections.

Results

(*A*) *Experiments in acutely splanchnicotomized dogs.*

Experiments were carried out in 3 dogs, whose splanchnic nerves were cut about 2 hours before the onset of experiments. The results are presented in Table I.

In Exp. 1, the adrenaline secretion rate before the first acetylcholine injection was 0.02 μg. per kg. of body weight per minute. On receiving acetylcholine it was accelerated and reached 0.30 μg. in the first 60-second

TABLE I

Effect of Hexamethonium on the Augmented Adrenaline Secretion of the Adrenal Gland Causable by Acetylcholine in Acutely Splanchnicotomized Dogs

No. of experiment	Body weight and sex	Adrenaline secretion rate (μg.) per kg. per minute					
		Before hexamethonium			After hexamethonium		
		Before acetylcholine	After acetylcholine (sec.)		Before acetylcholine	After acetylcholine (sec.)	
			0–60	60–120		0–60	60–120
1	13.5 kg. ♀	0.02	0.30	0.03	*	0.01	*
2	9.0 kg. ♂	*	0.34	0.03	0.03	0.03	0.01
3	6.6 kg. ♂	0.02	0.34	*	*	0.04	0.03

* Immeasurably small amount of adrenaline in adrenal venous blood.

period after the start of injection. In the second 60-second period it returned to 0.03 μg. After injection of hexamethonium the secretion rate was immeasurably small. By the second acetylcholine injection the adrenaline secretion rate was not increased, 0.01 μg. being estimated in the first 60-second period. And in the second 60-second period it was too small to be estimated.

In Exp. 2, the rate of adrenaline secretion before the first acetylcholine injection was immeasurably small. After the injection it increased and reached 0.34 μg. per kg. per minute in the first 60-second period. After hexamethonium the adrenaline secretion rate was 0.03 μg. By the second acetylcholine injection no appreciable increase in the secretion rate was induced, 0.03 μg. and 0.01 μg. being estimated in the first and the second 60-second period, respectively.

In Exp. 3, the adrenaline secretion rate before the first acetylcholine injection was 0.02 μg. per kg. per minute. After the injection it increased to 0.34 μg. in the first 60-second period. It was immeasurably small in the second 60-second period. After hexamethonium the adrenaline secretion rate was too small to be estimated. After the second acetylcholine injection the secretion rate was estimated as 0.04 μg. and 0.03 μg. in the first and the second 60-second period, respectively.

(*B*) *Experiments in chronically splanchnicotomized dogs*

Six dogs were used. In 3 dogs (Exps. 4–6) the splanchnic nerves were cut 2 weeks and in 3 others (Exps. 7–9) 3 weeks prior to experiments. The results are shown in Table II.

In Exp. 4, the pre-injection control secretion rate of adrenaline was 0.01 μg. per kg. per minute. On receiving acetylcholine, the adrenaline secretion rate increased to 0.28 μg. in the first 60-second period after the start of injection. And in the second 60-second period it decreased to 0.02 μg. After hexamethonium injection, the adrenaline secretion rate before the second acetylcholine injection was immeasurably small. After the acetylcholine injection the rate of adrenaline secretion increased and reached 0.19 μg. in the first 60-second period. Then it decreased to 0.03 μg.

Almost the same results were obtained in Exp. 5. The rate of adrenaline secretion before the first acetylcholine injection was 0.03 μg. per kg. per minute. After the injection of acetylcholine it increased and reached 0.38 μg. in the first 60-second period. In the next 60-second period it was measured as 0.10 μg. After application of hexamethonium the adrenaline secretion rate was too small to be estimated. By the second acetylcholine injection the secretion rate was increased to 0.14 μg. in the first 60-second period. It was 0.05 μg. in the second 60-second period.

In Exp. 6, the adrenaline secretion rate before the first acetylcholine

TABLE II

Effect of Hexamethonium on the Augmented Adrenaline Secretion of the Adrenal Gland Causable by Acetylcholine in Chronically Splanchnicotomized Dogs

No. of experiment	Body weight and sex	Weeks after splanchnicotomy	Adrenaline secretion rate (μg.) per kg. per min.					
			Before hexamethonium			After hexamethonium		
			Before acetylcholine	After acetylcholine (sec.)		Before acetylcholine	After acetylcholine (sec.)	
				0–60	60–120		0–60	60–120
4	8.9 kg. ♂	2	0.01	0.28	0.02	*	0.19	0.03
5	11.0 kg. ♀	2	0.03	0.38	0.10	*	0.14	0.05
6	9.9 kg. ♀	2	0.03–0.04	0.99	0.04	0.02	0.14	0.03
7	9.4 kg. ♂	3	0.03–0.04	0.44	0.11	0.01	0.26	0.06
8	7.5 kg. ♂	3	*	0.42	0.03	0.02	0.22	0.06
9	8.2 kg. ♂	3	0.02–0.03	0.82	0.08	0.02	0.20	0.09

* Immeasurably small amount of adrenaline in adrenal venous blood.

injection was 0.03–0.04 μg. per kg. per minute. By the first acetylcholine injection it was increased to 0.99 μg. in the first 60-second period. In the second 60-second period it returned to the pre-injection secretion rate. Hexamethonium was then injected. The adrenaline secretion rate before the second acetylcholine injection was 0.02 μg. After injection of acetylcholine it increased to 0.14 μg. in the first 60-second period. In the next 60-second period it decreased to 0.03 μg.

In Exp. 7, the rate of adrenaline secretion before the first injection of acetylcholine was 0.03–0.04 μg. per kg. per minute. After the injection it was accelerated and estimated as 0.44 μg. and 0.11 μg. in the first and the second 60-second period, respectively. After administration of hexamethonium and before the second acetylcholine injection, the rate of adrenaline secretion was 0.01 μg. After the second acetylcholine injection it increased, 0.26 μg. and 0.06 μg. being estimated in the first and the second 60-second period, respectively.

Similar results were obtained in Exp. 8. The adrenaline secretion rate before the first acetylcholine injection was immeasurably small. After injection of acetylcholine it increased to 0.42 μg. per kg. per minute in the first 60-second period. In the second 60-second period it was estimated at 0.03 μg. After administration of hexamethonium, acetylcholine injection was made again. Before the second acetylcholine injection the adrenaline secretion rate was 0.02 μg. After the injection of acetylcholine it increased and reached 0.22 μg. in the first 60-second period. In the next

60-second period it was estimated at 0.06 μg.

In Exp. 9, the adrenaline secretion rate before the first acetylcholine injection was 0.02–0.03 μg. per kg. per minute. By the injection of acetylcholine it was increased and reached 0.82 μg. in the first 60-second period after the start of injection. In the next 60-second period it was 0.08 μg. After hexamethonium and before the second injection of acetylcholine the adrenaline secretion rate was 0.02 μg. On injecting acetylcholine it increased and was estimated as 0.20 μg. and 0.09 μg. in the first and the second 60-second period, respectively.

Discussion

In Exps. 1–3, in which the section of the splanchnic nerves was made about 2 hours before the onset of experiments, the secretion rates of the first 60-second periods after the first and the second acetylcholine injection were 0.30–0.34 μg. and 0.01–0.04 μg. per kg. per minute, respectively. The ratio of the latter to the former was on the average about 8:100. In experiments of Yamashita[2] with the innervated adrenal gland, the average ratio was approximately 5:100. Thus it is evident that the effect of hexamethonium on the acutely denervated adrenal gland is almost the same as that on the innervated gland.

On the other hand, in Exps. 4–6, in which the splanchnic nerves were cut 2 weeks before experiments, the adrenaline secretion rates after the first and the second acetylcholine injection were 0.28–0.99 μg. and 0.14–0.19 μg., respectively. The mean ratio of the latter to the former was about 40:100. In Exps. 7–9, in which the splanchnic nerves were cut 3 weeks before experiments, the secretion rates after the first and the second acetylcholine injection were 0.42–0.82 μg. and 0.20–0.26 μg., respectively. The ratio of the latter to the former was on the average 45:100. Thus it is deducible that in the chronically denervated adrenal gland a considerable increase in the adrenaline secretion is causable by acetylcholine even after administration of hexamethonium.

It is concluded that the paralyzing effect of hexamethonium on the adrenal medullary response to acetylcholine which is not affected by the acute denervation of the adrenal gland, is reduced definitely, if not completely, by the chronical denervation.

Summary

Nine dogs, anesthetized with Evipan-sodium, were used in the experiments. The adrenal venous blood was collected by the lumbar route method of Satake *et al.* and its adrenaline content was estimated chemically by the method of Bloor and Bullen.

In dogs, whose splanchnic nerves were cut about 2 hours before the onset of the experiments, the adrenaline secretion rate was increased markedly by the intravenous injection of acetylcholine before administering hexamethonium. After hexamethonium, however, no definite increase in the secretion rate was elicited by acetylcholine.

In dogs, whose splanchnic nerves were cut 2 or 3 weeks before experiments, a considerable increase in the secretion rate was induced by injection of acetylcholine even after administering hexamethonium, although it was smaller than that before hexamethonium.

Thus it may be reasonable to conclude that the suppressive effect of hexamethonium on the adrenal medullary response to acetylcholine is definitely reduced by the chronical denervation of the adrenal gland, but not by the acute denervation.

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Effect of Hexamethonium on the Adrenaline-Secretory Response of the Adrenal Gland to Carbaminoylcholine

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It was demonstrated that the augmentation of adrenaline secretion of the adrenal gland causable by acetylcholine was greatly reduced by hexamethonium.[1]

Carbaminoylcholine was proved to produce an increase in the adrenaline secretion of the adrenal gland.[2] Moreover, Yamashita *et al.*[3] found that the adrenaline-secretory action of carbaminoylcholine was in its potency several times as strong as that of acetylcholine.

The present study was attempted to know whether the effect of carbaminoylcholine on the adrenaline secretion is reduced by hexamethonium.

Experimental

Methods

The experiments were performed in eight dogs, weighing between 8.0 and 17.5 kg., under Evipan-sodium anesthesia. In all experiments, the greater and lesser splanchnic nerves were cut on the left side through the lumbar route before the start of experiments. In order to collect the adrenal venous blood, a small cannula was inserted into the left adrenal vein according to the technique described by Satake *et al.*[4] All the chemicals were injected intravenously into the saphenous vein.

For the purpose of suppressing the muscarine-like action of carbaminoylcholine, atropine sulfate was given in a dose of 1 mg. per kg. of body weight, as was done in experiments of Yamashita *et al.*[3] and Suzuki *et al.*[5] After the control adrenal venous blood samples were collected, carbaminolylcholine chloride (Doryl, Merck) in a dose of 0.4 mg. per kg. was injected intravenously in 15 seconds. During the first and the second 60-second period after the start of carbaminoylcholine injection, the adrenal venous blood collections were made. After about half an hour, hexa-

methonium (Bistrium bromide, Squibb) in doses of 0.75 mg., 0.50 mg., or 0.25 mg. per kg. was injected intravenously. Again, after collecting control blood sample, carbaminolycholine in the same dose as before was injected and the collections of the adrenal venous blood were performed. The adrenaline content of the adrenal venous blood samples was estimated colorimetrically by the arseno-molybdic acid method of Bloor and Bullen.[6] Adrenaline (Merck) was used as the reference standard.

Results

The data are summarized in Table I

Hexamethonium in a dose of 0.75 mg. per kg. was injected in 4 dogs.

In Exp. 1, the adrenaline secretion rate before the first carbaminoylcholine injection was immeasurably small. By the injection, the rate of adrenaline secretion was increased remarkably and was estimated at 0.43 μg. per kg. of body weight per minute in the first 60-second period.

TABLE I

Effect of Hexamethonium on the Adrenaline Secretion of the Adrenal Gland in Response to Carbaminoylcholine

No. of experiment	Body weight and sex	Dose of hexamethonium (mg.) per kg.	Adrenaline secretion rate (μg.) per kg. per minute					
			Before hexamethonium			After hexamethonium		
			Before carbaminoylcholine	After carbaminoylcholine (sec.)		Before carbaminoylcholine	After carbaminoylcholine (sec.)	
				0-10	60-120		0-60	60-160
1	8.0 kg. ♂	0.75	*	0.43	0.02	0.01	0.01	*
2	8.6 kg. ♂	0.75	0.01	0.41	0.03	0.01	0.02	0.01
3	9.7 kg. ♂	0.75	0.01	0.35	0.08	*	0.02	0.02
4	10.8 kg. ♂	0.75	0.01	0.65	0.03	0.06	0.09	0.03
5	10.5 kg. ♀	0.50	0.01	0.32	0.06	*	0.06	0.02
6	12.5 kg. ♂	0.50	0.01	0.32	0.03	*	0.06	0.01
7	10.9 kg. ♀	0.25	0.04	0.57	0.09	0.02	0.24	0.03
8	17.5 kg. ♀	0.25	*	0.35	0.05	0.02	0.16	0.04

* Immeasurably small adrenaline content of the adrenal venous blood.

In the next 60-second period it was 0.02 μg. After the application of hexamethonium the rate of adrenaline secretion was 0.01 μg. By the second carbaminoylcholine injection the secretion rate was not increased. It was 0.01 μg. and immeasurably small rate in the first and the second 60-second period, respectively.

Similar results were obtained in Exp. 2. The adrenaline secretion

rate before the first carbaminoylcholine injection was 0.01 μg. per kg. per minute. After carbaminoylcholine injection, it was increased remarkably and reached 0.41 μg. in the first 60-second period. In the next 60-second period it was 0.03 μg. After hexamethonium the secretion rate was 0.01 μg. In the first and the second 60-second period after the second carbaminoylcholine injection it was 0.02 μg. and 0.01 μg., respectively.

In Exp. 3, the pre-injection control secretion rate of adrenaline was 0.01 μg. per kg. per minute. After the first carbaminoylcholine injection, the adrenaline secretion rate was increased markedly and reached 0.35 μg. in the first 60-second period. In the second 60-second period it was 0.08 μg. After hexamethonium the adrenaline secretion rate was too small to be estimated. The secretion rate after the second carbaminoylcholine injection was 0.02 μg. in the first as well as in the econd 60-second period.

In Exp. 4, the adrenaline secretion rate before carbaminoylcholine was 0.01 μg. per kg. per minute. On receiving carbaminoylcholine it was accelerated remarkably and reached 0.65 μg. in the first 60-second period. Then it decreased to 0.03 μg. After hexamethonium the adrenaline secretion rate was 0.06 μg. After the second carbaminoylcholine injection the adrenaline secretion rate was estimated at 0.09 μg. and 0.03 μg. in the first and the second 60-second period, respectively.

In Exps. 5 and 6, hexamethonium in a dose of 0.50 mg. per kg. was applied.

In Exp. 5, the rate of adrenaline secretion was increased by the first carbaminoylcholine injection from 0.01 μg. to 0.32 μg. per kg. per minute in the first 60-second period. In the next 60-second period it was measured as 0.06 μg. After hexamethonium injection, the secretion rate was immeasurably small. After the second carbaminoylcholine injection the rate of adrenaline secretion was 0.06 μg. and 0.02 μg. in the first and the second 60-second period, respectively.

The results of Exp. 6 were almost the same as those of Exp. 5. The adrenaline secretion rate before the first carbaminoylcholine injection was 0.01 μg. per kg. per minute. On receiving carbaminoylcholine the adrenaline secretion rate increased. It was measured as 0.32 μg. and 0.03 μg. in the first and the second 60-second period, respectively. After hexamethonium the secretion rate was immeasurably small. Then carbaminoylcholine was injected again. The adrenaline secretion rate was measured as 0.06 μg. and 0.01 μg. in the first and the second 60-second period.

In Exps. 7 and 8, hexamethonium was given in a dose of 0.25 mg. per kg.

In Exp. 7, the adrenaline secretion rate before the first carbaminoylcholine injection was 0.04 μg. per kg. per minute. It was augmented by

carbaminoylcholine and was measured as 0.57 μg. and 0.09 μg. in the first and the second 60-second period, respectively. The rate of adrenaline secretion after hexamethonium was 0.02 μg. Carbaminoylcholine was injected again. The adrenaline secretion rate was increased. It was 0.24 μg. and 0.03 μg. in the first and the second 60-second period, respectively.

In Exp. 8, the rate of adrenaline secretion was immeasurably small before the first carbaminoylcholine injection. After the injection it increased and reached 0.35 μg. per kg. per minute in the first 60-second. In the 60-second period it was 0.05 μg. After hexamethonium the secretion rate was 0.02 μg. After the second carbaminoylcholine injection it increased, 0.16 μg. and 0.04 μg. being measured in the first and the second 60-second period, respectively.

Discussion

In Exps. 1–4, hexamethonium was injected in a dose of 0.75 mg. per kg. Before the application of hexamethonium, the adrenaline secretion was increased by carbaminoylcholine, the maximum being estimated at 0.35–65 μg. during the first 60-second period. After hexamethonium a definite increase in the adrenaline secretion rate was not produced by the carbaminoylcholine injection. It was measured as 0.01–0.09 μg. in the first 60-second period. Calculating the ratio of the latter to the former in each case, it is 7:100 on the average. This indicates that hexamethonium, given in a dose of 0.75 mg. per kg., suppresses definitely the augmented adrenaline secretion causable by 0.4 mg. carbaminoylcholine per kg.

In Exps. 5 and 6, in which hexamethonium was given in a dose of 0.5 mg. per kg., the mean ratio is calculated as 19:100. In these cases the suppressive effect of hexamethonium on the adrenaline-secretory action of carbaminoylcholine was clearly demonstrated.

In Exps. 7 and 8, in which 0.25 mg. hexamethonium per kg. was given. The adrenaline secretion rate was increased definitely by carbaminoylcholine even after hexamethonium application, indicating that this dose of hexamethonium is not large enough for preventing the augmented adrenaline secretion causable by carbaminoylcholine.

Almost the same results were obtained in the acetylcholine-experiments of Yamashita.[1] In his experiments it was demonstrated that hexamethonium, given in doses of 0.50–1.0 mg. per kg., was capable of suppressing the augmented adrenaline secretion causable by 2.0 mg. acetylcholine per kg., whereas 0.25 mg. hexamethonium per kg. did not suppress it.

SUMMARY

Experiments were performed in eight dogs under Evipan-sodium anesthesia. The adrenal venous blood was collected by the cannula inserted into the adrenal vein according to the method of Satake *et al.* and was estimated for adrenaline by use of the arseno-molybdic acid method of Bloor and Bullen.

By the injection of carbaminoylcholine in a dose of 0.4 mg. per kg. the adrenaline secretion rate of the adrenal gland was markedly increased and was estimated at 0.32–0.65 μg. per kg. per minute in the first 60-second period after the start of injection.

Hexamethonium in doses of 0.50–0.75 mg. per kg. was applied and this was followed by the injection of carbaminoylcholine in the same dose as before. At this time, the augmentation of adrenaline secretion was small, 0.01–0.09 μg. being estimated there.

Thus, it may be concluded with definiteness that hexamethonium is capable of reducing the augmented adrenaline secretion of the adrenal gland causable by carbaminoylcholine.

The author is grateful to Prof. T. Suzuki and Dr. K. Yamashita for their advice and help given in the present investigation.

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A Method for Measuring the Peripheral Blood Flow by Para-aminohippurate

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Owing to the recent advances in medicine, we have now methods for estimating the volume of the blood flow in many important organs.

As to the blood flow in peripheral regions two kinds of method were reported: the one consists in applying the plethysmography, by which Barcroft *et al.*,[1] Goetz[2], Burch[3] and Ishikawa *et al.*[4] and others have studied, and the other consists in using the radioactive isotope Na^{24}, by which Smith *et al.*[5] and Kety[6][7] have studied.

By the method of plethysmography the volume of the blood flow usually can not be estimated numerically, and in many reports by this method, for example in that of Ishikawa *et al.*[4], numerical volume of the blood flow has not been demonstrated and has been only compared with normal range qualitatively, not quantitatively. This can be said about the report of Smith *et al.*[5] also.

And so it is thought that some problems lie unsolved in the estimation of the blood flow in peripheral regions, so we will report here a method for measuring the peripheral blood flow quantitatively by PAH, although it also contains one assumption, which must be clarified in future.

Theoretical Consideration

Fig. 1 is a scheme expressing the arteries and veins in one region of a body.

When C represents the PAH concentration in the arteries in the region under PAH equilibrium and F the volume of the plasma flowing into the region in one minute, that is, the plasma flow, then the volume of the PAH, which flows into the region from time o to t is expressed as follows:

$$\int_0^t FCdt = F\int_0^t Cdt \qquad (1)$$

On the other hand, the flow out of the region occurs through veins

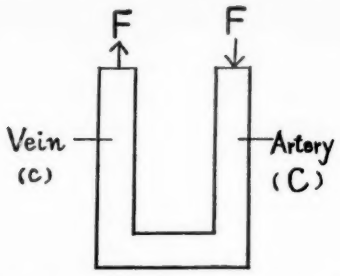

Fig. 1. A scheme of the arteries and veins in a region of a body. C and c represent the PAH concentration in the arteries and veins respectively and F represents the plasma flow per minute in the region.

and lymphatics. Because the PAH concentration can be thought to be approximately equal in the veins and lymphatics in the region under PAH equilibrium as explained later, the volume of the PAH, which is carried away out of the region is expressed as follows, when c respresents the PAH concentration in the veins.

$$\int_0^t Fcdt = F\int_0^t cdt \qquad (2)$$

According to Schwartz[8] PAH enters into tissue cells, but as we[9] reported previously, the PAH, which entered into tissue cells, is thought not to flow easily out of the cells into interstitial fluid, and therefore the PAH in the cells can be thought to have no relation to PAH equilibrium.

Now, when the plasma volume in the arteries and veins (the plasma volume in the veins includes in this report the volume of the fluid in the lymphatics) and the volume of the interstitial fluid in the region are represented by A, V and G respectively, and the PAH concentrations in the arteries, veins and interstitial fluid in the region at time o are represented by Co, c_0 and C_{Go} respectively and at time t by C, c and C_G, the following equation can be obtained from (1) and (2) by Fick's principle.

$$F\left\{\int_0^t cdt - \int_0^t Cdt\right\} = A(C_0 - C) + V(c_0 - c) + G(C_{G0} - C_G) \qquad (3)$$

As reported previously[10][11] the PAH concentration in arteries is smaller than that in veins and the logarithms of each concentration are expressed by two parallel straight lines against time as drawn in Fig. 2, and when the direction co-efficient of these lines are represented by $-\alpha$, each straight line can be expressed by such equations as written in Fig. 2. Therefore

$$\left.\begin{aligned} C &= C_0 10^{-\alpha t} \\ c &= c_0 10^{-\alpha t} \end{aligned}\right\} \qquad (4)$$

Therefore

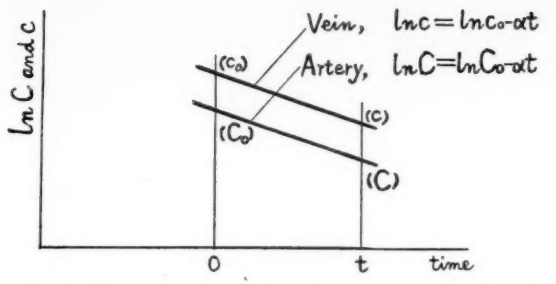

Fig. 2. Relation of logarithms of PAH concentrations in artery and vein to time. C_0 and c_0 represent the PAH concentration in artery and vein respectively at time o, and C and c at time t. And $-\alpha$ represents the direction co-efficient.

$$\frac{C}{c} = \frac{C_0}{c_0}$$

That is, the ratio of the PAH concentration in arteries to that in veins is kept constant at each time. Under PAH equilibrium PAH is excreted in the kidneys and the PAH concentrations in arteries and veins decrease with time, and so in order to keep equilibrium, PAH is thought to continue to flow into veins from interstitial fluid corresponding to the difference between the PAH concentrations in veins and interstitial fluid. So, because the ratio of the PAH concentration in arteries to that in veins is constant at each time, the PAH concentration in interstitial fluid must have a constant ratio to that in arteries and/or veins. And so the logarithm of the PAH concentration in interstitial fluid must make a straight line against time, the direction co-efficient of which is also $-\alpha$. Therefore

$$\ln C_G = \ln C_{G0} - \alpha t$$

Therefore

$$C_G = C_{G0} 10^{-\alpha t} \qquad (5)$$

From (3), (4) and (5)

$$F\left\{\int_0^t c_0 10^{-\alpha t} dt - \int_0^t C_0 10^{-\alpha t} dt\right\} = AC_0(1-10^{-\alpha t}) + Vc_0(1-10^{-\alpha t}) + G C_{G0}(1-10^{-\alpha t}) = (1-10^{-\alpha t})(AC_0 + Vc_0 + GC_{G0}) \qquad (6)$$

And $\int_0^t 10^{-\alpha t} dt = \dfrac{1-10^{-\alpha t}}{\alpha \log_e 10}$, so (6) becomes as follows:

$$F.\frac{(1-10^{-\alpha t})(c_0 - C_0)}{\alpha \log_e 10} = (1-10^{-\alpha t})(AC_0 + Vc_0 + GC_{G0}) \qquad (7)$$

Because the PAH concentration in arteries and consequently in veins decreases with time due to the excretion of PAH in the kidneys, PAH, as explained above, flows continuously from interstitial fluid into veins to

keep equilibrium, and the fact, that PAH concentration in veins is usually greater than that in arteries considerably, means that a large volume of PAH has moved into veins out of interstitial fluid, that is, that PAH can move freely and rapidly between capillaries and interstitial fluid, and therefore the PAH concentration in interstitial fluid will be almost equal to or only a little greater than that in veins, and the difference between these two will be very slight, compared with the difference between the PAH concentrations in veins and arteries.

On the other hand, comparing the volume of interstitial fluid with that of the plasma in arteries, the former is much greater than the latter, and according to Hernandez-Peón[12] the volume of interstitial fluid occupies 13.2% of body weight, and that of plasma 4.3%. If this rate can be applied to the region in question, the volume of the interstitial fluid in the region G is three times the volume of the plasma in the region and therefore it can be thought to be greater than three times the volume of the plasma in the arteries in the region A, perhaps to be equal in approximation to six or seven times A or more.

And as explained above, the difference between the PAH concentrations in the interstitial fluid and veins will be much smaller than that between the veins and arteries in the region, and comparing these differences with each other at time o, $C_{G0}-c_0$ will be much smaller than c_0-C_0, and perhaps the latter may be assumed to be six or seven times the former or more. Therefore, even if we assume that the following formula will exist in approximation, there may not occur a great error.

$$A(c_0-C_0) \fallingdotseq G(C_{G0}-c_0)$$

Therefore

$$AC_0+GC_{G0} \fallingdotseq Ac_0+Gc_0 \qquad (8)$$

From (7) and (8)

$$F\cdot\frac{(1-10^{-\alpha t})(c_0-C_0)}{\alpha \log_e 10} = (1-10^{-\alpha t})(A+V+G)c_0$$

According to Schwartz[8] and Schwartz *et al.*[13] mannitol-, inulin- and thiosulfate-spaces correspond to the volume of the extracellular fluid and PAH-space is greater than those spaces. This means that a part of PAH enters into tissue cells and the other part is distributed in all the extracellular fluid. As explained above, the PAH which entered into tissue cells has no relation to PAH equilibrium, and so the PAH-space which has relation to it can be thought to be equal to the volume of the extracellular fluid. Although (A+V+G) is the PAH-space relating to the PAH equilibrium, it will correspond in approximation to the volume of the extracellular fluid ECF in the region. Therefore the following formula can be obtained.

$$\frac{F}{ECF} = \frac{\alpha c_0 \log_e 10}{c_0 - C_0} = \frac{2.303 \alpha c_0}{c_0 - C_0} \qquad (9)$$

$\frac{F}{ECF}$ in (9) is the volume of plasma flow per 1 cc. of extracellular fluid in the region per minute. So the volume of the plasma flow per 100 cc. of extracellular fluid in the region per minute is represented by the following formula.

$$\frac{F}{ECF} \times 100 = \frac{2.303 \alpha c_0}{c_0 - C_0} \times 100 \qquad (10)$$

Experimental Method

The data, which were used in the present study, are those, which had been gotten on 32 normal and hypertensive individuals in the two studies reported previously as "A Method of Measuring the Volume of the Circulating Blood in the Lungs and the Minute Volume by Means of Sodium Para-aminohippurate"[10] and "On the Measurement of the Respective Volumes of the Circulating Blood in Arteries and in Veins."[11] Using these data we calculated the volume of the plasma flow per 100 cc. of extracellular fluid per minute from (10). In these previous experiments we took three blood samples from femoral artery as well as from antecubital vein between 30 and 50 minutes after intravenous injection of 2000 mg. of PAH as previously reported, and estimating the PAH concentration in these six samples we got two parallel straight lines, which the logarithms of the PAH concentrations in the artery and vein made against time.

Because the PAH concentration is equal in all arteries, the value of $\frac{F}{ECF} \times 100$, which is calculated from (10) is the volume of the plasma flow per minute per 100 cc. of extracellular fluid in the forearm of the side, on which the venous blood was taken for samples. And we calculated the renal plasma flow, adopting 88% as extraction rate according to Warren *et al.*[14]

Experimental Results and Discussion

In Table I and II we present the values of the plasma flow per 100 cc. of extracellular fluid in a forearm, renal plasma flow and, in cases where blood pressure has been estimated, the values of blood pressure and mean arterial pressure (diastolic pressure $+\frac{1}{3}$ pulse pressure), on 22 normal and 10 hypertensive individuals. In this report we included two cases No. 24 (blood pressure 146/94, mean arterial pressure 111) and No. 25 (blood pressure 140/94, mean arterial pressure 109) in hypertension, which had

TABLE I

Values of Peripheral Plasma Flow, Renal Plasma Flow, Blood Pressure and Mean Arterial Pressure

$\frac{F}{ECF} \times 100$ represents the plasma flow per 100 cc. of extracellular fluid in forearm per minute, and RPF, BP and MAP represent the renal plasma flow, blood pressure and mean arterial pressure respectively, in normal individuals.

No.	Age	Sex	Diagnosis	$\frac{F}{ECF} \times 100$ (cc.)	RPF (cc.)	BP	MAP
1	30	♂	normal	18.8	587		
2	23	"	"	16.0	563		
3	29	"	"	8.7	451		
4	37	"	"	20.5	554		
5	41	"	"	10.1	679		
6	30	"	"	8.7	241		
7	27	"	"	15.1	676		
8	27	"	"	8.0	622		
9	29	"	"	11.9	870		
10	24	"	"	12.1	877		
11	22	♀	"	14.2	635		
12	22	"	"	25.3	693		
13	29	"	"	22.6	661		
14	35	♂	"	18.2	706	116/78	91
15	40	"	"	15.6	657	120/70	87
16	43	"	"	13.2	875	132/80	97
17	26	"	"	14.9	681	124/60	81
18	29	"	"	13.5	708	124/70	88
19	45	"	"	17.3	498	126/76	93
20	38	"	"	19.1	413	120/0	40
21	62	"	"	16.7	602	112/80	91
22	41	"	"	14.8	807	130/80	97
		Mean		15.2			

been reported previously as normal.

As shown in tables the plasma flow per 100 cc. of extracellular fluid in the forearm per minute is 8.0–25.3 cc. (mean 15.2 cc.) in normal and 11.9–36.1 cc. (mean 18.3 cc.) in hypertensive individuals. Barcroft *et al.*[1] reported 2–7 cc. as the blood flow per 100 cc. of tissue of the forearm estimated by plethysmography. We will compare it with ours.

According to Hernandez-Peón[12] the volume of the extracellular fluid corresponds to 17.5% of body weight in approximation. And so, if this rate can be applied to the forearm in question, 100 cc. of the extracellular

TABLE II

Values of Peripheral Plasma Flow, Renal Plasma Flow, Blood Pressure and Mean Arterial Pressure

In hypertensive individuals. For abbreviations see Tab. I.

No.	Age	Sex	Diagnosis	$\frac{F}{ECF} \times 100$ (cc.)	RPF (cc.)	BP	MAP
23	50	♂	Essential Hypertension	16.4	831		
24	54	"	"	11.9	731	146/94	111
25	55	"	"	15.2	609	140/94	109
26	57	"	"	14.5	414	210/120	150
27	58	"	"	23.1	430	180/90	120
28	59	"	"	18.4	697	184/80	114
29	44	"	"	36.1	718	166/90	115
30	65	"	"	16.2	424	176/100	125
31	54	"	"	15.5	579	160/100	120
32	53	"	"	15.9	481	155/90	111
		Mean		18.3			

fluid corresponds approximately to 570 cc. of the tissue of the forearm Consequently, when we convert our values into those for the plasma flow per 100 cc. of the tissue of the forearm, we get as plasma flow 1.4–4.4 cc. (mean 2.7 cc.) in normal and 2.1–6.3 cc. (mean 3.2 cc.) in hypertensive individuals. When we convert these values into blood flow, we get 2.3–7.3 cc. (mean 4.5 cc.) as blood flow per 100 cc. of the tissue of the forearm in normal and 3.5–10.5 cc. (mean 5.3 cc.) in hypertensive individuals in approximation, although we have not estimated hematocrit values. Our values on normal individuals are equal in approximation to those reported by Barcroft *et al.* as 2–7 cc.

Then we will compare our results with Kety's. Kety[6][7] injected Na^{24} into gastrocnemius and calculated the value for k from the following formula,

$$k = \frac{\log C_1 - \log C_2}{0.4343(t_2 - t_1)}$$

where C_1 and C_2 equal counts by Geiger-Müller counter per minute at time t_1 and t_2 respectively. And on the other hand, k is represented theoretically by the following formula

$$k = \frac{mF_V}{S} + \frac{nF_L}{S}$$

where S represents sodium space in a small unit mass of the tissue, into which Na^{24} has been injected, F_V and F_L the respective venous and lym-

phatic outflow per minute, and m and n are constants with values between o and 1. According to him k, that is, the clearance constant, although not a rigid measure of total blood flow, is an accurate and quantitative measure of the effectiveness of the local circulation including not only blood flow but other mechanisms responsible for local homeostasis. In tissues where diffusion occurs rapidly and is not a limiting factor, the clearance constant approximates the blood flow.

The values for k in 8 cases reported by him are between 0.033–0.066 (mean 0.050). Because Na^{24} is distributed only in extracellular fluid and it is so diffusible that m and n may be equal in approximation to 1, we can assume the values for k equal in approximation to the plasma flow per 1 cc. of extracellular fluid per minute in gastrocnemius, and so the plasma flow per 100 cc. of extracellular fluid per minute is 3.3–6.6 cc. (mean 5.0 cc.) in gastrocnemius, one third our values in forearm. (It must be emphasized that Kety has not performed such a calculation for the reason mentioned above.) This difference between the values obtained by our method and calculated by us from Kety's results seems to depend upon the difference of the region, at which measurement was done, because it seems natural that the blood flow will differ in different organs or tissues.

Sancetta *et al.*[15] have reported 0.0232–0.4226 cc. as the rate of blood flow in the finger per 5 cc. of part per minute determined by the venous occlusion technique of Cranley *et al.*[16] in 8 normal individuals. When these values are converted in the rate of blood flow in the finger per 100 cc. of part per minute, we get 0.46–8.5 cc. (mean 4.8 cc.). Our values for the blood flow per 100 cc. of the tissue in the forearm per minute in normal individuals are 2.3–7.3 cc. (mean 4.5 cc.) as mentioned above, and both values are approximately equal.

By the method reported by Smith *et al.*[5] and Ishikawa *et al.*[4] numerical volume of the blood flow can not be obtained.

Comparing our results with those of the authors above mentioned it is supposed that the theory underlying this method will be correct and it can be applied clinically.

Between blood pressure and the blood flow in forearm the relation $R = \frac{P}{F}$ must exist, where R, F and P represent the peripheral resistance, the blood flow and the pressure difference between the mean arterial pressure and the venous pressure, respectively. The venous pressure is said to vary considerably in different individuals, and in hypertension it is usually within normal limits. In these experiments we have not estimated the venous pressure in antecubital vein and also hematocrit values, so we can not know the accurate pressure difference and the volume

of the blood flow, but, because the venous pressure will be very small, when compared with the mean arterial pressure, and the ratio of the plasma flow to the blood flow will be almost constant, the ratio of the mean arterial pressure to the plasma flow can be thought to be proportional to the peripheral resistance. We expected that this ratio would be greater in hypertensive individuals than in normal, but such a relation could not be found. Because the values of the plasma flow in the forearm in hypertensive individuals are slightly greater than normal as shown in tables, there results consequently no increase in the peripheral resistance in the forearm of the hypertensive in spite of increase in the mean arterial pressure. For this, two reasons can be guessed: 1) that in the forearm of the hypertensive there is no constriction of arterioles more than the normal or 2) that, although the arterioles in the forearm constrict to some extent in hypertensive individuals, the constriction of the arterioles in other organs or tissues is more increased than in the forearm and the blood flow in those organs or tissues of greater resistance will be decreased, resulting in increase in the blood flow in the forearm, because it is an accepted fact that the cardiac output is usually of normal value in hypertension. In case No. 27 the renal plasma flow is 430 cc. and the plasma flow in forearm 23.1 cc., and the latter seems to be relatively greater, compared with the former. So it can be supposed that in this case the blood flow in the forearm may be increased passively owing to the decrease in the renal plasma flow, against which the resistance in the kidneys would presumably have been increased. But the extraction rate of PAH in hypertensive individuals, whose kidneys have been injured, is expected to be smaller than in normal, and if so, the real renal plasma flow will be greater than the calculated value. In case No. 29, in which the renal plasma flow and the plasma flow in the forearm are both of great value, it is presumed that the stroke volume and cardiac output will be greater than normal, resulting in hypertension and increase in the plasma flow both in kidneys and forearm, but because we have not estimated it, we can not conclude.

Finally, we did not estimate hematocrit values and venous pressure and the cases studied are few in number, so we can conclude nothing about the hemodynamics in hypertension in this study.

This method is based on the assumption, that in the forearm the relation $AC_0+Vc_0+GC_{G0} \fallingdotseq (A+V+G)c_0$ will exist in approximation, and therefore the blood flow can be estimated by this method in any organ or tissue, where the above mentioned assumption will exist, and this method can be applied clinically for estimating the blood flow in peripheral organs or tissues. But it must be clarified in future, to what extent the assumption $AC_0+Vc_0+GC_{G0} \fallingdotseq (A+V+G)c_0$, that is, that the mean concentration of PAH in the extracellular fluid is approximately equal to the PAH

concentration in the veins, will exist in organs or tissues in question.

Summary

A method for measuring the volume of the peripheral blood flow by using PAH was reported. The values of the plasma flow per 100 cc. of extracellular fluid in forearm per minute was 8.0–25.3 cc. (mean 15.2 cc.) in 22 normal individuals and 11.9–36.1 cc. (mean 18.3 cc.) in 10 hypertensive, and this method is supposed to be able to be applied clinically for estimating the blood flow in organs or tissues, where the assumption, that the mean concentration of PAH in the extracellular fluid is approximately equal to the PAH concentration in the veins, can exist.

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Electrophoretic Studies of Liver Protein

Report II. Studies on Liver Protein in Surgical Gastric Diseases

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The writers have previously reported on electrophoresis applied to the study of liver protein in this Journal[1], and here we will report on the liver protein fractions in surgical gastric diseases as analyzed by our method.

Experimental

Methods

For preparing the extract of the liver tissue protein (called simply liver protein hereunder) from liver specimens, the procedure for electrophoresis and identification of the fractions, the methods previously described[1] were followed.

In discussing the behavior of the liver protein fractions, the state of the liver functions come into the question. For testing the liver functions in the experimental subjects, the serum icterus index, Bromsulphalein test and Takada's serum test were applied. Cases with the icterus index below 10 were evaluated as normal, those with 10–20 as mild and over 20 as severely troubled in liver function; the cases with Bromsulphalein test (30 min. value) below 5 percent as normal, those with 5–20 percent as with mild and over 20 percent as with severe liver troubles. The cases with positive reaction in Takada's serum, were evaluated as severely disturbed in their liver function. In general evaluation, the cases showing positivity in any one of the above three tests were deemed as having mild disturbance (+) and those with more than one positive reactions as severely disturbed in their liver function (++).

Results

I. *Liver protein fractions in the mild cases of gastric diseases*

Of the 108 cases tested, two of chronic gastritis and six of gastric ulcer showed more than 4 million in red cell count, more than 80 percent in hemoglobin content (Sahli), hematocrit value of 42–43 percent, normal level of liver function and were in good general conditions. These eight cases will be taken up first. In these cases only Ringer's solution or 5 percent glucose solution (500–1000 cc) was administered but no blood transfusion was applied before operation.

The measured contents of the liver protein fractions in these cases are given in Table I. The ranges and the mean values of 4.0–7.1 percent

TABLE I

Liver Protein Fraction Values in Mild Cases

Case	Fraction values (%)				
	Alb.	α-Gl.	β-Gl.	ζ-Gl.	γ-Gl.
Gastritis	4.0	16.2	47.5	21.1	11.2
Gastritis	5.2	16.1	46.5	19.3	12.9
Gastric ulcer	5.9	17.9	46.3	19.9	10.0
Gastric ulcer	5.9	19.0	45.2	20.8	9.1
Gastric ulcer	6.1	17.3	45.9	17.8	12.9
Gastric ulcer	6.2	14.0	49.2	20.5	10.1
Gastric ulcer	6.4	19.4	48.1	18.5	7.6
Gastric ulcer	7.1	16.6	48.9	18.4	9.0
Average	(4.0~7.1) 5.8	(14.0~19.4) 17.0	(45.2~49.2) 47.1	(17.8~21.1) 19.4	(7.1~12.9) 10.7

and 5.8 percent of albumin, 14.0–19.4 percent and 17.8 percent of α-globulin, 45.2–49.2 percent and 47.1 percent of β-globulin 17.8–21.1 percent and 19.4 percent of ζ-globulin and 7.6–12.9 percent and 10.7 percent of γ-globulin may be taken as the control value under normal liver function.

II. *Liver Protein fractions in cases of gastric diseases*

The results of electrophoresis obtained with six cases of chronic gastritis, 44 cases of gastric or duodenal ulcer and 50 cases of gastric cancer, compared with the values cited above, the α-globulin value showed little change, the difference nearly always consisting in the changes in the albumin, β-, ζ- and γ- globulin values; so the β-globulin value was chosen as the main criterion in classifying the cases into five types, as follows:

Type I. Cases with all the values of the fractions within the control range.

Type II. Those with subnormal β-globulin values and high ζ-globulin values, but the other fractions within the control range.

Type III. Those with low β- but high γ-globulin values, with the other fractions within the control range.

Type IV. Those with lowered β-globulin but raised albumin and γ-globulin values.

Type V. Those with lowered β-globulin and raised albumin values, the other fractions remaining within the control range.

(1) *Liver protein fractions in cases of gastritis and gastric ulcer* In the 50 cases of gastritis and ulcers, the liver protein fraction picture belonged to Type I in 26 cases, to Type II in four cases, to Type III in seven cases, to Type IV in nine and Type V in four cases. The maximum, the minimum and the mean values in individual cases by the Types were as summarized in Table II.

Among the 26 cases of Type I, two had pyloric stenosis, three had severe anaemia, and one had perforation, but excepting these, the other 20 cases showed excellent general conditions and no complicating symptoms. In these cases, all the tests for the liver function showed results well within the normal ranges.

The four Type II cases were also free of complications and showed good general conditions, but their β-globulin content showed the low values of 34.7–39.3 percent, averaging 37.1 percent and their ζ-globulin value was always above 22.8 percent, showing the high mean of 28.9 percent.

TABLE II

Liver Protein Fraction Values in Gastritis-Ulcer Cases

Type of cases	Number of cases	Fraction values (average, %)				
		Albumin	α-Glob.	β-Glob.	ζ-Glob.	γ-Glob.
Type I	26	5.6 (3.5~ 7.8)	18.5 (13.8~20.0)	46.2 (44.5~50.2)	19.6 (16.8~21.3)	10.1 (7.2~12.5)
Type II	4	7.4 (3.1~ 9.2)	16.7 (11.1~20.7)	37.1 (34.7~39.3)	28.9 (22.8~40.3)	9.9 (8.1~10.8)
Type III	7	7.2 (4.8~ 8.4)	15.1 (9.4~21.5)	35.1 (22.2~39.9)	18.5 (13.3~21.7)	24.1 (14.0~43.4)
Type IV	9	11.7 (10.0~13.6)	14.6 (8.8~19.0)	31.3 (25.0~40.7)	18.3 (14.3~22.7)	24.1 (14.6~31.4)
Type V	4	14.6 (11.2~17.7)	19.0 (17.3~20.5)	39.1 (36.1~41.3)	18.0 (15.8~20.7)	9.3 (7.9~11.5)

Of the seven Type III cases, five were suffering from hemorrhagic ulcers and showed seriously deteriorated general conditions, while another had pyloric stenosis and the remaining one had pulmonary tuberculosis in complication, both showing bad general conditions. In all these cases the β-globulin value was very low (mean value: 35.1%) but the γ-globulin

value very high (mean value: 24.1%). Especially, in two of the cases, the β-globulin value was reduced to 22.2 percent and 26.0 percent and the γ-globulin value risen to 43.4 percent and 38.2 percent, respectively, showing a very peculiar tendency.

In these seven cases of Type III, the β-globulin value averaged by 12.0 percent below the control (or dropped by 25.4% in rate) and the γ-globulin value averaged 13.4 percent above the control (or risen by 125.2% in rate). In these cases, however, all were found to be free of liver disturbances, except one which showed a slight disturbance in the liver function.

In Fig. 1. are shown the representative liver protein electrophoretic patterns of cases with hemorrhagic gastric ulcer belonging to Type III.

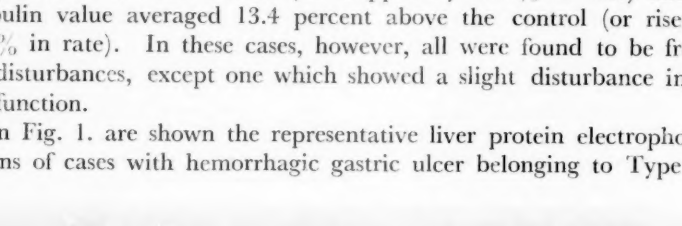

Fig. 1. Electrophoretic patterns of Type III cases with hemorrhagic ulcer.

Of the nine cases belonging to Type IV, one showed good general conditions, but five had hemorrhagic ulcer, two had pyloric stenosis and one had lung tuberculosis in complication, these eight showing deteriorated general conditions. Especially, in the cases of hemorrhagic ulcer, the red cell count was lowered below 3.3 million, the hemoglobin content to below 70 percent (Sahli) and the serum protein to below 6.0 g./dl., indicating serious conditions.

In all these cases, the albumin content always rose above 10.0 percent, averaging 11.7 percent and cases with 13.4 percent and 13.6 percent of this value were observed, while the β-globulin value was below 40.7 percent in all cases, averaging 31.3 percent, especially notably low individual values of 25.0, 27.2 and 27.7 percents were observed. The γ-globulin value was supernormal, always exceeding 14.6 percent and averaging 24.1 percent. In particularly high cases, the value amounted to 27.2, 30.6 and 31.4 percents.

The mean value of albumin in these Type IV cases was higher by 5.9 percent than the control (or risen to 101.7%), that of β- and γ-globulins was lowered by 15.8 percent (or fallen by 33.5% in rate) and risen by 13.4 percent (or raised by 125.5% in rate), respectively. Among these nine cases, however, slight liver failure was observed in only two cases.

Of the four Type V cases, the general conditions were good in only one case, two of the other cases having hemorrhagic ulcers and one having pyloric stenosis.

In these cases, the albumin value always rose above 11.2 percent, averaging 14.6 percent, in one case rising to 17.7 percent, and the β-globulin value stood between 41.3 percent and 36.1 percent, averaging 39.1 percent below the mean control value.

In these four cases of Type V, the mean albumin value was risen by 8.8 percent (or risen by 151.7%), and the β-globulin value was lowered by 8.0 percent (or dropped by 16.9%) from the mild case. In all these cases, the liver function tests showed normal values, except in one case with slight liver failure.

Among the above gastritis and gastric ulcer cases, 50 in total, Type I liver protein fraction picture was observed in 26 cases, that of Type II in four cases, of Type III in seven cases, of Type IV in nine cases and of Type V in four cases; 24 of the 30 Types I and II cases (80%) showed good general conditions and 18 of the 20 Types III, IV and V cases (90%) had complications.

(2) *Liver protein fractions in cases of gastric cancer* Among the 50 cases of gastric cancer, the liver protein fraction picture belong to Type I in 21 cases, to Type II in four cases, to Type III in 12 cases, to Type IV in seven and to Type V in six cases. The individual maximum, the minimum and the mean values of the liver protein fractions by the Types were as summarized in Table III.

In the 21 cases of Type I, including three with pyloric stenosis and two with highly advanced cancer, all showed good general conditions. A highly advanced cancer means that the tumors were widely disseminated, accompanying ascites or wide metastasis, the cancer was so advanced that no other operation than mere exploratory laparotomy or total gastrec-

tomy was performed.

The four Type II cases were all with highly advanced cancer, including one that had to be subjected to total gastrectomy and another case for whom we could undertake only exploratory laparotomy. The liver function was slightly disturbed in two cases.

In these cases the β-globulin value was always reduced, though moderately (38.7%–41.6%, averaging 40.0%) but the ζ-globulin value rose always above 22.7 percent averaging 29.0 percent, and in particular, went up extremely to high values in two cases (33.3% and 37.2%).

Of the 12 Type III cases, two showed good enough general conditions, but in the other 10 cases, the general conditions were badly deteriorated, of which four were subjected to total gastrectomy and two to exploratory laparotomy. The liver function was slightly disturbed in three cases.

In these cases, the β-globulin value was always low (43.6%–36.1%, averaging 40.9%) but the γ-globulin value was high (13.5%–20.2%, averaging 16.8%).

The mean β-globulin value in these cases was reduced by 7.2 percent (or dropped by 13.1% in rate) and the mean globulin value was raised by 6.1 percent (or by 57.0% in rate) as compared with the values of the mild cases.

Of the seven cases of Type IV, one was rather good in general conditions, but in the others, the cancer was highly advanced, including one case with hemorrhagic cancer and four that had to undergo total gastrectomy.

TABLE III

Liver Protein Fraction Values in Gastric Cancer Cases

Type of cases	Number of cases	Fraction values (average, %)				
		Albumin	α-Glob.	β-Glob.	ζ-Glob.	γ-Glob.
Type I	21	6.5 (4.2~ 8.0)	17.6 (13.5~19.3)	45.7 (45.3~47.2)	19.6 (17.0~20.6)	10.6 (7.0~11.8)
Type II	4	5.2 (2.5~ 7.7)	15.9 (14.4~20.3)	40.0 (38.7~41.6)	27.0 (22.7~37.2)	9.9 (6.8~12.3)
Type III	12	5.9 (3.1~ 7.5)	16.2 (12.3~21.8)	40.9 (36.1~43.6)	20.2 (17.0~22.2)	16.8 (13.5~20.2)
Type IV	7	10.9 (10.2~12.1)	16.0 (12.4~20.3)	37.8 (31.6~42.5)	17.2 (13.2~19.3)	18.1 (14.4~26.7)
Type V	6	14.9 (11.4~20.3)	19.6 (17.0~22.5)	36.3 (31.2~39.1)	19.9 (17.7~22.0)	9.3 (7.0~11.2)

In these cases the albumin value was always raised (10.2%–12.1%, averaging 10.9%), the β-globulin value was lowered (42.5%~31.6%,

averaging 37.8%) and the γ-globulin was found risen (14.4%–26.7%, averaging 18.1%). In the case with hemorrhagic cancer, in particular, the albumin value registered 11.2 percent, the β-globulin, value 33.6 percent and the γ-globulin value 26.7 percent, manifesting a very peculiar tendency.

In comparison with the mean value of mild cases, the mean albumin value in these seven cases was higher by 5.1 percent (or by 87.9% in rate), that of β-globulin was lower by 7.4 percent (or by 19.7% in rate) and that of γ-globulin higher by 7.4 percent (or 69.1% in rate). The liver function was slightly disturbed in one case only.

In the seven cases of Type V, the cancer was highly advanced and the general conditions were also deteriorated. Two of them were operated upon for total gastrectomy and two others for exploratory laparotomy. The liver function was slightly disturbed in three cases.

In these cases, the albumin value was always over 11.4 percent averaging 14.9 percent and in one case the particularly high level of 20.3 percent was observed, while the β-globulin value was always low (31.2%–39.1%, averaging 36.3%).

In comparison with the values of mild cases, the mean albumin value was higher by 9.1 percent in absolute value (or by 156.8% in rate) and the mean β-globulin value was lower by 10.8 percent (or by 22.9% in rate).

In the above 50 cases of gastric cancer, 21 showed liver protein fraction picture of Type I, four that of Type II, 12 that of Type III, seven that of Type IV and six that of Type V. Sixteen of the 25 cases (64%) of Types I and II showed good general conditions, while 23 of the 25 cases (92%) of Types III, IV and V had complications or highly advanced cancer.

III. *Comparison of liver protein fraction picture in gastritis-ulcer cases and gastric cancer cases*

The values in the two groups of such cases are summarized in Table IV.

The Type I cases occupied a bare majority of 26 among 50 gastritis-ulcer cases (52%), but among the 50 cancer cases 21 of Type I cases were found (42%), a smaller number than among the former.

The Type II cases accounted for 8 percent only in both the groups.

The Type III cases represented 24 percent (12 among 50) in the cancer group, while in the gastritis-ulcer cases 14 percent (7 among 50) were of this type; thus, among the former, the cases with low β-globulin and high γ-globulin were more frequent than among the latter.

The Type IV cases were somewhat more frequent in the gastritis-ulcer group (9 or 18%) than among the gastric cancer cases (7 or 14%), while of Type V cases, the reverse was the case (4 or 8% in the former and 6 or 12% in the latter groups).

TABLE IV

Comparison of Liver Protein Fraction Picture in Gastritis-ulcer Cases and Gastric Cancer Cases

Case ↓ Type→	I	II	III	IV	V
Gastritis-ulcer (50 cases)	26 (52.0%)	4 (8.0%)	7 (14.0%)	9 (18.0%)	4 (8.0%)
Gastric cancer (50 cases)	21 (42.0%)	4 (8.0%)	12 (24.0%)	7 (14.0%)	6 (12.0%)

Discussion and Conclusions

Recently, with the advance in filter-paper electrophoresis, studies on electrophoretic studies on tissue protein have increased, but there is no report on a clinical study of the liver protein, so far as we known.

We have herein presented the findings on the albumin, and the α-, β-, ζ- and γ-globulin contents in the protein extracted from the liver of 108 surgical gastric disease cases, as fractioned by electrophoresis and identified by the methods described in Report I.

Of the 108 cases, eight showed very good general conditions, their red cell count, hemoglobin content and hematocrit value were all within the normal range, and their liver function was also normal; we took these mild gastric disease cases as controls in this study. Of their liver protein fraction, albumin was present only in a very small quantity (mean value: 5.8%) while the globulins occupied a very large part (mean value: 94.2%) and among these, β-globulin was most abundant.

Demling *et al.*[2] divided the cellular components into three parts and by paper-electrophoretically fractionating of the protein in these parts, they found the mean values of 4.87 percent of albumin, 15.66 percent of α-globulin, 55.67 percent of β-globulin and 23.78 percent of γ-globulin. Their method of extracting protein was not the same as ours, but their findings on the smallness of the albumin content and the great predominance of globulins may be deemed as similar to our results cited above.

Luck[3] and Urban[4] also proved that the liver protein consists in the greatest part of globulin, by the biochemical method.

In our study on the protein fractions of 100 cases except for the eight above, we attempted a classification into five types with the changes in the β-globulin content as the main criterion.

Among the 50 cases of each of gastritis or gastric ulcer and of gastric cancer, 24 of the 30 Types I and II cases of the former (80%) and 16 of 25 Types I and II cases of the latter (64%) showed good general conditions.

But the Types III, IV and V cases were in the majority cases with

bad general conditions, having hemorrhage or pyloric stenosis in complication (18 of 20 cases or 90% among the gastritis-ulcer cases and 16 of 25 cases or 64% of gastric cancer cases).

Thus, among the cases of gastric diseases, those with the liver protein fraction picture within the range of the control values of mild cases were generally good in general conditions, while those with the picture of reduced β-globulin and augmented γ-globulin and/or albumin were usually cases with complications or advanced diseases.

Demling *et al.*[2] in their experiments with rats, have demonstrated that β-globulin in the liver protein was markedly reduced in the cases with general disturbances due to poisons. We found a similar tendency in our clinical cases.

It must be noted that such a tendency is more marked in the cases of hemorrhagic ulcer than in those with gastric cancer.

Ohi[5] and Le Veen[6] report that bleeding in the stomach causes several functional and morphological disturbances to the liver than peripheral bleeding. We found no marked liver disturbances by our clinical liver function tests in our cases with gastric hemorrhage, but in our liver protein electrophoresis, we found a tendency specific to them, so that from the angle of liver protein we can make inference on the specific effect of gastric hemorrhage.

In the last place, we found that the cases belonging to our Type I with their liver protein fraction contents within the range of the control values were somewhat more frequent among the gastritis-ulcer cases (52%) than among the gastric cancer cases (42%), while these belonging to the Types III, IV and V with the β-globulin value lower, the γ-globulin and/or the albumin value higher than the controls were somewhat more numerous among the latter (50%) than among the former (40%), this tendency being most marked among the Type III cases (24% among the gastric cancer and 14% among the gastritis-ulcer cases). The slightly higher frequency of IV cases among the gastritis-ulcer cases may be due to the five hemorrhagic ulcer cases among the nine Type IV cases.

Summary

By means of our electrophoretic method applied to analyse the liver protein, as reported in Part I of this series, we studied the liver protein fractions in eight cases of chronic gastritis and 50 cases each of gastric or duodenal ulcer and of gastric cancer. The results obtained were as follows:

1. The liver protein in two cases of chronic gastritis and six of gastric ulcer, but only in mild grade as diagnosed clinically, was found to contain only a very small quantity of albumin (5.8% on the average), the remainder being all globulins, β-globulin being particularly abundant (47.1% on

the average).

2. Of the severe cases of gastric diseases, the cases showing the liver protein fraction values within the range of the above mild cases were generally of good general conditions, but those with lowered β-globulin, raised γ-globulin and/or albumin value were usually cases with complications or advanced disease.

3. The liver protein fraction values were more frequently within the values of mild cases in gastritis-ulcer cases than in gastric cancer cases, and the β-globulin value was lowered, the γ-globulin and/or the albumin value raised more frequently among the gastric cancer cases.

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Electrophoretic Studies of Liver Protein

Report III. Studies on Liver Protein in Surgical Biliary Tract Diseases

By

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The results obtained with cases of some surgical diseases of the biliary tract are presented in this third report.

Experimental

Method

The method of recording the electrophoretic patterns of liver protein and the clinical liver function tests were the same as described in Reports I[1] and II[2] of this series.

Results

Our subjects comprised 17 cases with cholelithiasis, 12 with non-calculous cholecystitis, two with cancer of the bile duct and one each with hepatitis, hepatic abscess and liver cirrhosis, 35 cases in total.

The cases were classified into the five types as described in Report II by the results of electrophoretic analysis of their liver protein.

I. *The liver protein fraction values in the tested subjects*

Of the 35 cases of biliary tract diseases, six showed the liver protein fraction picture of Type I, three of Type II, nine of Type III, eight of Type IV and nine of Type V. The maximum, minimum and mean values in the groups of all the types are given in Table I.

The six cases of Type I consisted of three cholelithiasis cases and one each of hepatitis, liver cirrhosis and bile duct cancer cases. In the three latter cases, the liver function was severly affected and the general conditions were anything but good, but the fraction values remained in the range of the control values.

The Type II cases comprised two cases with cholelithiasis and one

with bile duct cancer and highly disturbed liver function. The β-globulin value in these cases was obviously subnormal (44.6%–36.1%, averaging 40.3%), while the ζ-globulin value was high (24.7%–27.3%, averaging 26.4%).

TABLE I

Liver Protein Fraction Values in Biliary Tract Disease Cases

Type of cases	Number of cases	Fraction values (average, %)				
		Albumin	α-Glob.	β-Glob.	ζ-Glob.	γ-Glob.
Type I	6	7.2 (5.3~ 8.5)	15.0 (13.5~19.2)	48.2 (47.0~51.4)	21.3 (20.8~21.8)	7.9 (2.3~11.2)
Type II	3	6.2 (4.3~ 7.4)	17.6 (15.1~21.3)	40.3 (36.1~44.6)	26.4 (24.7~27.3)	9.5 (8.7~10.5)
Type III	9	6.4 (1.6~ 8.4)	16.3 (12.5~20.3)	37.9 (29.1~43.5)	17.9 (13.9~22.6)	21.4 (14.0~34.6)
Type IV	8	10.9 (9.0~15.2)	17.1 (15.0~20.9)	35.8 (28.5~43.0)	16.2 (13.2~20.9)	19.0 (13.5~26.2)
Type V	9	13.8 (10.7~23.1)	19.0 (15.4~21.6)	38.2 (29.6~43.6)	19.1 (15.7~21.3)	10.9 (8.1~12.7)

The nine Type III cases consisted of two cases with non-calculous cholecystitis with intact liver function and seven cholelithiasis cases of whom four had slight liver disturbances; all these nine cases showed good general conditions. In these Type III cases, the β-globulin value was low (37.9% on the average) and the γ-globulin value was high (21.4%). In one case each with cholelithiasis and non-calculous cholecytsitis free of liver disturbances, the very low β-globulin values of 31.5 percent and 29.1 percent and the very high γ-globulin values of 25.7 percent and 34.6 percent were observed, respectively. The mean β-globulin value in the Type III cases was lower by 9.2 percent in absolute value or 19.5 percent in rate and the mean γ-globulin value was higher by 10.7 percent of by 100 percent in rate than the mean values in the controls.

Of the eight cases of Type IV, one showed heavy and two slight disturbances in their liver function, but the others had no liver disturbance and showed good general conditions. In these cases, the albumin value was always high (above 9.0% averaging 10.9%), the β-globulin low (averaging 35.8% and coming down individually to the extreme low of 28.5% and 30.4%) and the γ-globulin value high (averaging 16.7% and rising individually to the extreme high of 24.9% and 26.2%). In these Type IV cases, the mean albumin value was higher by 5.1 percent or by 87.9 percent in rate, the β-globulin value lower by 11.3 percent or 24 percent in rate and the γ-globulin value higher by 6.0 percent or 56.1 percent in rate than the mean values in the mild cases. The representative

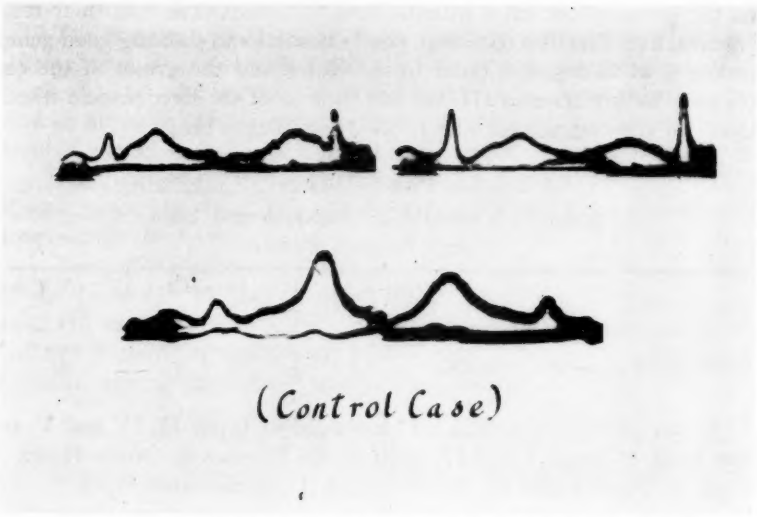

Fig. 1. Representative electrophoretic patterns of liver protein in Type IV cases of non-calculous cholecystitis.

patterns of protein fractions in non-calculous cholecystitis cases belonging to Type IV is given in Fig. 1.

Of the nine Type V cases, mild and severe liver disturbances were observed in two cases each respectively but no liver failure could be detected and the general conditions were good in the remaining five cases. In these cases, the albumin value was high above 10.7 percent and averaging 13.8 percent, in a case rising to the high of 23.1 percent, while the β-globulin value was always below 43.6 percent, averaging 38.2 percent, in some individual cases falling to the low levels of 32.3 percent and 29.6 percent. Among these Type V cases, three with non-calculous cholecystitis were free from liver disturbances. The mean albumin value of these nine cases was 8.0 percent above mild cases or risen by 137.9 percent and the mean β-globulin value 8.9 percent below mild cases or reduced by 18.9 percent in rate.

Of the above detailed cases, five of the nine cases (56%) belonging to Types I and II showed good general conditions and 15 of the 26 cases (58%) belonging to the other types also were of good general conditions and free from liver disturbances.

II. *Relation between the liver function and the liver protein fraction picture*

When the above 35 cases of diseases in the biliary tract are classified

into the group of the cases without liver disturbances so that their results of clinical liver function tests were nearly normal and showing good general conditions as Group I without liver failures and the group of the cases with liver failure (Group II), the distribution of the liver protein fraction picture in the two groups was as shown in Table II.

TABLE II

Relation between Liver Function and Liver Protein Fraction Picture

Cases ↓ Type→	I	II	III	IV	V	Sum.
Group I (Cases without liver failures)	2	1	5	5	5	18
Group II (Cases with liver failures)	4	2	4	3	4	17

Of the 18 cases in Group I, 15 belonged to Types II, IV and V, only three being of Types I and II, while of the 17 cases in Group II, six belonged to Types I and II, the remaining 11 to the other types.

Thus, in the cases of diseases of the biliary tract, about three-fourths of the total cases (26 or 74.3%), whether with or without liver disturbances, showed the liver protein fraction picture of lowered β-globulin and raised γ-globulin and/or albumin value.

III. *Comparison of the liver protein fraction picture in the biliary tract diseases and gastric diseases*

The subject values in the present cases of biliary tract diseases and those of gastric diseases, as reported in Report II, are given in Table III.

The rate of the cases belonging to Type I was far smaller among the 35 biliary tract disease cases (6 or 17.2%) than among the 100 gastric disease cases (47 or 47%).

TABLE III

Comparison of Liver Protein Fraction Picture in Biliary Tract Disease and Gastric Disease Cases

Case ↓ Type→	I	II	III	IV	V
Biliary tract disease (35 cases)	6 (17.2%)	3 (8.5%)	9 (25.7%)	8 (22.9%)	9 (25.7%)
Gastric disease (100 cases)	47 (47.0%)	8 (8.0%)	19 (19.0%)	16 (16.0%)	10 (10.0%)

The frequency of Type II cases was small (around 8%) in both groups.

The frequency of Type III cases was 25.7 percent (9 cases) among the biliary tract disease cases, being a little higher than among the gastric

disease cases (19 cases or 19%).

The frequency of Type IV cases was higher among the biliary tract disease cases (8 cases or 22.9%) than among the gastric disease cases (16 cases or 16%), i.e., cases with lowered β-globulin value, raised γ-globulin and or albumin values were more frequent among the cases of the biliary tract diseases.

The number of Type V cases was nine (25.9%) in the biliary tract disease cases and 10 (10%) in the gastric disease cases, being far more frequent in the former.

Thus, of the biliary tract disease cases 74.3 percent (26 cases) belonged to the Types III, IV and V, while of the gastric disease cases only 45 percent (45 cases) were of these types; that is, the frequency of cases with lowered β-globulin, raised γ-globulin and/or albumin value were far more frequent among the biliary tract disease cases.

Discussion and Conclusions

Reports on the decrease of liver protein at the time of liver disturbances have been published by Elman[3], Areil[4], and Yuasa[5]. Sato *et al.*[6] confirmed the decrease in clinical cases and experimental animals and reported on it in a previous issue of this Journal, but there has been no report on the liver protein fractions in such cases, except for the one by Demling *et al.*[7]

In this study, the writers have analyzed the liver protein sampled from surgically operated cases of biliary tract diseases into the five fractions indentifiable by the electrophoretic method and studied the relation of the fraction contents to the liver function etc.

In the 35 cases of surgical biliary tract diseases, Type I and Type I cases comprised the minority (9 cases or 25.7%), while the remaining 4.3 percent (26 cases) belonged to the Types III, IV and V.

As to the liver function, there was found no relation between the function and the liver protein fraction picture, for low β-globulin and high γ-globulin and/or albumin value was found even in the cases without liver disturbances, being particularly marked in the eight non-calculous cholecystitis cases without liver disturbances; especially, a very specific fraction picture of extremely low β-globulin value (29.1%) and high γ-globulin value (34.6%) was notable. Such specific cases was similar to the finding in hemorrhagic ulcer cases as described in Report II.[2]

Demling *et al.*[7] found decrease of β-globulin in the liver protein in the case of experimental liver disturbances, and stated that the behavior of β-globulin in the liver protein may be taken as an index of the liver function.

In our study, we found not rarely cases with lowered β-globulin and

raised γ-globulin and/or albumin value among the cases of biliary tract diseases but showing good general conditions and no liver disturbances in clinical tests, as well as in the cases with liver disturbances. This finding was in coincidence with that in the severe cases of hemorrhagic ulcers with no liver disturbances perceptible by clinical tests; this suggests that there may be some latent liver disturbances not detected by routine liver function tests, in the cases of biliary tract diseases, viewing from the angle of liver protein.

In comparison with gastric disease cases, we found a far more higher rate of Types III, IV and V cases among the biliary tract disease cases.

Summary

Using our method for obtaining electrophoretic patterns of the liver protein, the liver protein fraction in 35 cases of surgical biliary tract diseases was studied. The results obtained were as follows.

1. Among the cases of biliary tract diseases, not to speak of the cases with liver disturbances detected by clinical function tests, even in the cases free from such disturbances and showing good general conditions, lowered β-globulin value and raised γ-globulin and/or albumin value in their liver protein could be frequently observed.

2. In comparison with the previously reported cases of gastric diseases, the liver protein fraction picture with lowered β-globulin value and raised γ-globulin and/or albumin value was more frequent among the biliary tract disease cases.

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The Color-Receptive Mechanism of the Human Retina as Revealed by Zeta-Time Curves

By

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It was found by Motokawa[1)2)] that the time-course of electrical excitability of the human eye following a brief illumination was characteristic of the wavelength of the light used. The excitability was measured by a test pulse of 0.1 sec. in duration with electrical phosphenes as an index. The increase of excitability over the resting level of the dark-adapted eye was expressed in percentage of the latter and denoted by ζ (zeta). Since then the zeta-time curve was utilized for investigations of color vision as well as psychological effects including induction. In view of the fundamental importance of this phenomenon for physiological studies of vision, we tried in the present investigation to obtain standard curves for monochromatic lights of equal energy. Fortunately we had a well-trained subject available in our laboratory, who had given consistent results throughout a variety of experiments carried out in our laboratory.

Method

Monochromatic lights of various wavelengths were produced by the use of a series of interference filters (Lange & Co.), the transmission bands of which were less than 20 mμ in width. The monochromatic lights so obtained were made equal with respect to energy by means of suitable neutral tint filters. The measurement of energy was carried out by a surface-thermopile (Kipp-Sonen & Co.) consisting of 80 elements and a sensitive galvanometer. By this method the distribution of energy could be made uniform within experimental error of about 3 per cent.

The energy distribution of white light can differ greatly depending upon its source. The white light used so far in our laboratory was almost the same as the white light from the source A of the ICI which is defined as an incandescent lamp with a tungsten filament of 2848°K. The energy distribution of the source A is represented by a broken curve marked by open circles in Fig. 1. As can be seen in this figure, the energy increases steadily with wavelengths.

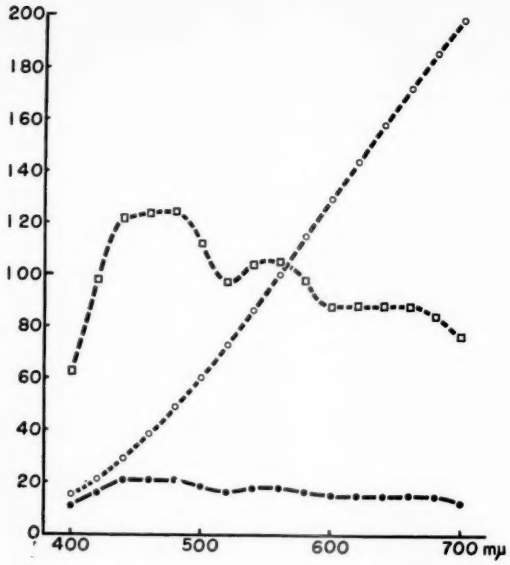

Fig. 1. Energy distribution of white light from source A (broken curve marked by open circles) and that of white light from source C (broken curve marked by squares). Total energy was same for both. Continuous curve marked by dots shows energy distribution of white light C actually used in comparison with white light A.

The distribution of energy is more uniform for day light which can be imitated by the source C of the ICI. The energy distribution of the source C is shown by a broken curve marked by squares in the same figure. The source C may be prepared with two kinds of fluid filters combined with the source A. The constitutions of the filters C_1 and C_2 are as follows:

$CuSO_4 \cdot 5H_2O$	3.412 g.	C_1
Mannite $(C_6H_8(OH)_6)$	3.412 g.	
Pyridine (C_5H_5N)	30.0 ccm.	
Dissolved in water to	1000.0 ccm.	

$CoSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$	30.580 g.	C_2
$CuSO_4 \cdot 5H_2O$	22.520 g.	
H_2SO_4 (s 1.835)	10.0 ccm.	
Dissolved in water to	1000.0 ccm.	

The thickness of each filter should be 1 cm.

In the present experiment the color-temperature of the incandescent lamp used was made equal to 2848°K by adjusting the voltage and measuring the color-temperature with an optical pyrometer.

Zeta-time curves were obtained for the so prepared two kinds of white light, A and C, and compared with each other.

The procedure to measure the electrical excitability was the same as described in a previous paper.[3] The measurements of zeta-time curves for monochromatic lights were carried out at the fovea and at a retinal area on the temporal meridian 20° from the fovea.

Results and Discussion

Zeta-time curves for monochromatic lights

In Fig. 2 two sets of zeta-time curves, one for the fovea and the other for the periphery, are illustrated. At the fovea the maximum of the curve lies at about 1, 2 and 3 seconds for red lights of 675–640 mμ, green

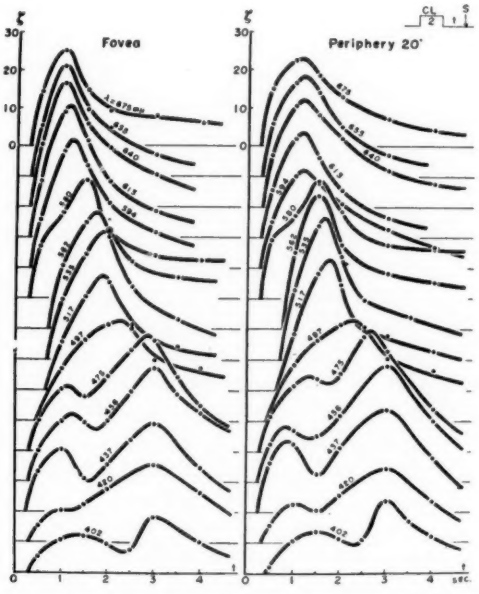

Fig. 2. Zeta-time curves caused by monochromatic lights of equal energy. Experimental procedure is shown in inset on right top where C.L., t and S dentoe colored light, time interval and electric shock of 0.1 sec. respectively. Baseline of each curve is shown by a horizontal line which is separated from the baseline of adjacent curve by 10 in zeta-value.

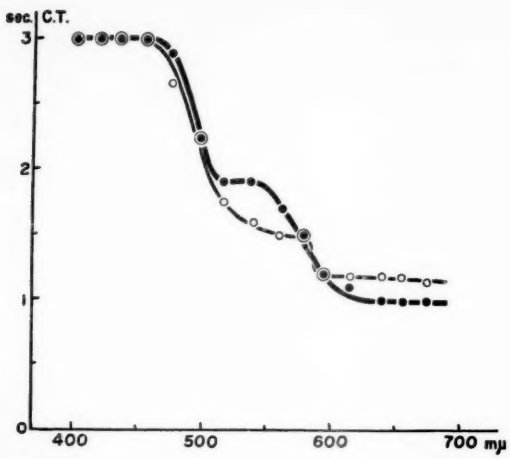

Fig. 3. Crest tims (C.T.) as a function of wavelengths. Continuous curve marked by dots refers to fovea and that marked by open circles to 20° from fovea.

lights of 535–517 mμ and blue lights of 475–402 mμ respectively. For blue and violet lights another small maximum can be seen at 1 second. Between red and green lights a gradual shift of the maximum can be seen, and also between green and blue lights another shift of the maximum is visible. In order to survey this relation more quantitatively, the crest time or the time from the end of the colored stimulus to the maximum of the zeta-time curve was plotted as a function of wavelengths in Fig. 3. As is shown by a continuous curve marked by dots, the crest time does not vary steadily with increasing wavelengths, but three distinct steady levels of crest time can be seen. This is in agreement with a previous finding by Motokawa[4]. The highest one of these levels concerns blue lights, the middle one green lights and the lowest one red lights. The transition from one level to the next is rather abrupt, especially between the green and blue lights. This fact may be correlated with the fact that the red, green and blue are dominant colors in foveal vision. In the red and green regions the crest time remains unchanged as the wavelength changes. Therefore the capacity for hue discrimination must be low in these regions. This inference coincides with the results obtained in the experiments on hue discrimination by Steindler[5], Laurens and Hamilton[6] and others. For the same reasons the capacity of hue discrimination must be high in the intermediate regions between red and green and between green and blue, where the rate of change in crest time is higher, and in

reality it was shown that in these regions hue discrimination is excellent in normal subjects.

In the hue discrimination curve as studied by the previous investigators there is a region of relatively high hue discriminability between blue and violet parts of the spectrum, although in these regions the crest time is steady.

As to the hue discrimination for these regions it should, however, be taken into account that two peaks appear in the zeta-time curves for the lights of these regions. The crest time of the first small peak coincides with that of the zeta-time curve for red light, and this fact may be correlated with the psychological fact that violet is reddish blue. As can be seen in Fig. 2, the relative magnitude of the first peak against that of the second becomes greater in agreement with the increasing subjective redishness towards the violet end of the spectrum. It is almost obvious that the relative magnitudes of both components as represented by both peaks are important for hue discrimination. Therefore, we should not relate the crest time of the dominant maximum alone to the capacity of hue discrimination.

Generally speaking, the zeta-time curve represents an envelope of a few component curves, and it seems more legitimate to assume that all the components more or less take part in hue discrimination. From this point of view, Motokawa and Isobe[7] attempted to account for the hue discrimination curves of normal and color-defective subjects in terms of components, R. Y, G and B, such as exemplified in Fig. 4.

In the parafoveal and peripheral regions of the retina red light appears orange, and in accordance with this quality of sensations, no curve with a crest time of 1 second has been obtained for lights of 675–640 mμ. The crest time of the curves for these lights was 1.2 seconds which is equivalent to the crest time of the curve for orange light at the fovea. As is shown by the continuous curve marked by open circles in Fig. 3 no steady level of crest time can be found over the green region. If one attempt to find any steady levels of crest time in the periphery of the retina, they are the levels of 1.2, 1.5 and 3 seconds. This would correspond to the known prevalence of yellow and blue sensations in the periphery.

Spectral response curves

The experiments stated above show that sensations of red, yellow, green and blue are characterized by the crest times of about 1, 1.5, 2 and 3 seconds respectively. If the ordinates at 1 second of respective zeta-time curve are plotted against wavelengths, a response curve for the red process will be obtained. Those for the yellow, green and blue processes will similarly be obtained by using ordinates at 1.5, 2 and 3 seconds respectively. The set of the response curves so obtained for the fovea is

illustrated in Fig. 4 where notations R, Y, G and B refer to the red, yellow, green and blue processes respectively. The curve R shows a dominant maximum at about 620 mμ and a second maximum at about 460 mμ. The other curves have only a single maximum, and the location of the maximum characterizes each process.

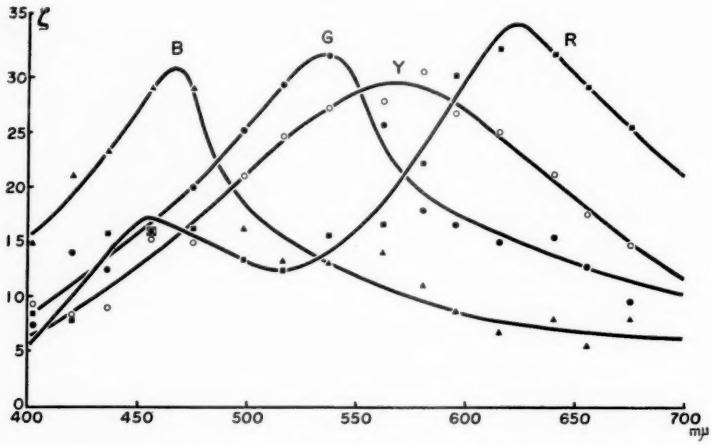

Fig. 4. Response curves for R (red), Y (yellow), G (green) and B (blue) processes constructed from set of curves on left of Fig. 2.

It is to be noted that the curve Y is lowest of all. If the intensity of light were lower, this process would not be manifest. In the previous paper by Motokawa[2] only three processes R, G and B were visible as if it conformed to the trichromatic theory. At the fovea and for a small visual angle the process Y was shown to be negligibly small[7].

For the construction of response curves it is profitable to measure zeta values—say at 1 second—for the whole range of wavelengths successively within one session. By this method a much smooth response curve than those illustrated in Fig. 4 will be obtained. The latter were obtained indirectly by way of zeta-time curves which were obtained day after day, so that the response curves based on these zeta-time curves were more irregular because of daily variations.

Zeta-time curves for white lights from sources A and C

The intensity of the white light A was measured at a circular patch placed at a distance of 30 cm. in front of the subject's eye. The patch was 1° in visual angle. The white light C was prepared in such a manner that the two filters mentioned above were placed just before the patch, the light source being A. The intensity was expressed in values measured

before the filters. The energy distribution of the white light C as measured behind the filters is represented by the continuous curve marked by dots in Fig. 1. One will notice a great difference in energy between white lights A and C.

The white light A appeared yellowish to the subject in comparison with the white light C. Curious to say, the subject reported that the white light C appeared as bright as the white light A, although the total energy of the former was decidedly smaller than that of the latter.

Zeta-time curves for white lights A of varying intensities are represented by broken curves in Fig. 5, where those for white lights C are shown

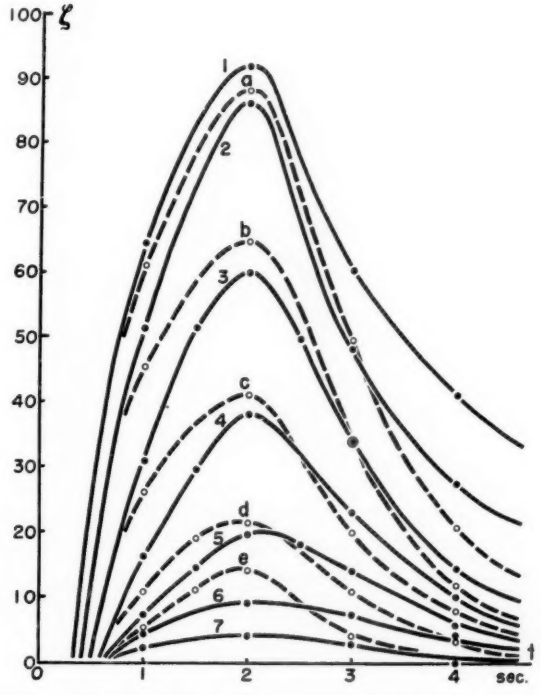

Fig. 5. Zeta-time curves for white lights A (broken curves) and C (continuous curves). Intensities for a, b, c, d and e were 10,000, 1,000, 100, 10, and 1 lux respectively. Intensities as measured before filters for 1, 2, 3, 4, 5, 6 and7 were 56,000, 5,600, 560, 56, 5.6, 0.56 and 0.056 lux respectively.

by continous curves. All of them have a maximum at about 2 seconds in agreement with the previous data. However, there is a marked difference between both kinds of curves concerning their forms. The continuous

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curve is lower at the premaximal part than the broken one to be compared, but higher at the postmaximal part, and this characteristic indicates the less yellowish and more bluish property of the white light C.

In Fig. 6 the maximal amplitudes of the zeta-time curves of both sorts are plotted as ordinates against the intensities as abscissae. It is to be noted that the intensities of white lights C given in this diagram refer to the values measured before the filters. Despite this situation, the data

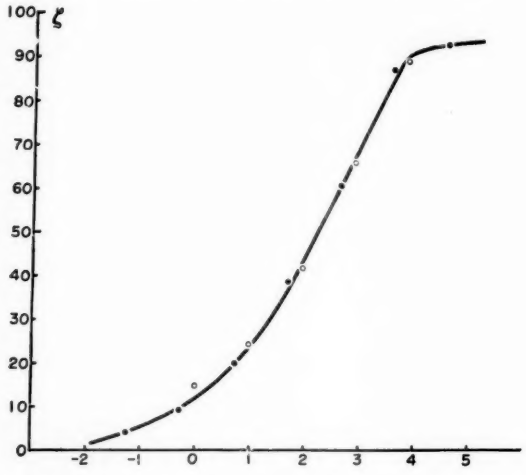

Fig. 6. Zeta-values at 2 seconds as a function of intensities of white lights A and C. Intensities of white lights C refer to values measured before filters. Abscissae log intensity (lux).

concerning both kinds of white light can be represented by a sigmoid curve common to both. This finding indicates that the filters used for the source C little affected the maximal amplitude of the zeta-time curve. This fact may be correlated with the similar brightness sensations evoked by both kinds of white light.

Curves of induction

Retinal induction caused by various colored lights was investigated by Motokawa[8] and Onoki[9]. In the present experiment more systematic measuremens were carried out on the same subject as served in the preceding experiment of zeta-time curves. On the left side of Fig. 7 curves of direct induction are illustrated, which were obtained by illuminating the fovea with a monochromatic light and then with a white test light. Both stimuli lasted for 2 sec. and were separated by an interval of 2 sec. The measurements of the electrical excitability were done at various times

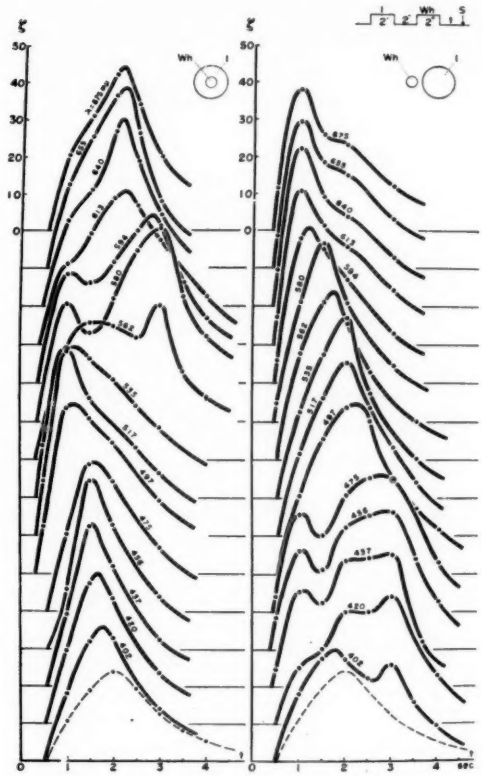

Fig. 7. Curves of direct induction (left) and those of indirect induction (right). Sequence of procedure and patches used are shown in insets where notations I, Wh, t, S stand for inducing colored light, white test light, time interval and electric shock respectively. Zeta-time curves for white test light are shown at bottom. Intensity of inducing light was 10,000 lux as measured before filter and that of white test light 50 lux.

from the end of the white test light. The broken curves at bottom are the zeta-time curves for the white test light alone.

The curve of direct induction is, in character, complementary to the curve which would be obtained for the colored light alone. The meaning of the complementarity of curves is as follows: As yellow and blue are complementary colors and cause zeta-time curves with a crest time of 1.5 sec. and 3 sec. respectively. Therefore a curve with a crest time of 1.5 sec. may be regarded as complementary to a curve with a crest time of 3 sec.

On the right side of Fig. 7 curves of indirect induction are shown. In this experiment the white test light illuminated a point just outside the area preilluminated by the colored light. The curve is, in character, complementary to the curve of direct induction caused by the same colored light.

Summary

The color processes caused by monochromatic lights of equal energy were investigated and expressed in terms of zeta-time curves which indicate the time-course of electrical excitability following a brief illumination.

1. Comparison was made on zeta-time curves obtained at the fovea and at 20° from the fovea.

2. Zeta-time curves for two kinds of white light from sources A and C of the ICI were compared.

3. Curves of direct and indirect induction were investigated at the fovea.

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Diet and Experimental Diabetes

I. Influence of Diet on Carbohydrate Metabolism in Normal Rats

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There have been a numerous trials to produce experimentally diabetes mellitus, such as administration of alloxan[1], extract of pituitary gland[2], thyroid[3], adrenal cortical steroid[4] and glucose[5], since Mering and Minkowski[6] reported experimental diabetes mellitus by pancreatectomy in dogs.

These pathogeneses in animals, however, are considered to be different from that in man from many standpoints, whereas it is worth to notice such factors related to diet or obesity.

Houssay and Martinez[7] reported that the occurrence of diabetes mellitus was observed in early period when the subtotally pancreatectomized animals were fed with the high lard diet.

In 1956, diabetes mellitus was induced in two dogs partially pancreatectomized and fed with high-protein high-fat diet by Takaoka and coworkers[8].

Recently Kotake and coworkers[9] observed that the rats showed hyperglycemia about one hundred and seventy days after the feeding with high-protein high-fat diet.

We experimented to observe the influence on the carbohydrate metabolism of rats fed with different diets.

Experimental

Materials and Method

One hundred and five normal male rats of the Wistar strain were used for this experiment. All animals were divided into seven groups and fed with different diets for two months as shown in Table I.

The changes of body weight, sugar in urine and fasting blood sugar were estimated during whole experimental period. Two months after the introduction of experimental feeding, all animals were subjected to glucose tolerance test after twelve fasting hours, in which 20% solution

TABLE I

Composition of Diet

Figures express weight proportions to total of 100, excepting those showing caloric values

Component	Diet group						
	I Standard	II High-protein (Isocal.)*	III High-protein (High-cal.)	IV High-fat (Isocal.)*	V High-fat (High-cal.)	VI High-protein high-fat (Isocal.)*	VII High-protein high-fat (High-cal.)
Carbohydrate	70	57	61	24	46	10	35
Protein	12	25	22	18	12	36	25
Fat	8	8	7	48	32	44	30
Vitamin & salt	10	10	10	10	10	10	10
Calories per day	82	82	92	82	107	82	105

Note: Carbohydrate: Irish potato flour. Protein: casein. Fat: lard. Vitamin and salt: Ebios, McCollum salt and cod liver oil in proportion of 4:4:2 by weight.

* With equal calories to the standard diet.

of glucose was given orally in amount of 2.0 gm. per kg. body weight. 0.05 cc. of blood was taken from the tail vein and the blood sugar level was estimated by Asari's modified method[10] of Fujita and Iwatake's micro-method[11]. In glucose tolerance tests, blood was taken in every 30 minutes for three hours.

Sugar in urine was checked by Testape (Lilly).

Few days after glucose tolerance test, every five rats in each dietary group were sacrificed for the pathohistological studies.

The free acetone value of blood and glucose uptake of diaphragm were estimated by Shimazono's modified method[12] of Greenberg and Lester[13], and Gemill's method[14], respectively.

Results

Change in body weight

Increase in body weight was observed in all dietary groups, especially remarkable in the group of high-protein high-fat diet.

Fasting blood sugar

Fasting blood sugar of normal male rats of the Wistar strain was 69 to 132 mg%, mean value being 109.6 mg%.

The blood sugar level remained in the normal range in all groups, none of them showing persistent hyperglycemia.

Urine sugar

Positive urine sugar was found in none of animals throughout whole experimental period.

Glucose tolerance test two months after the introduction of experimental feeding

The normal glucose tolerance curve is shown in Figure 1. These blood sugar levels are the mean values of twenty one normal male rats, showing the similar curve with that in man, reaching the peak in 30 to 60 minutes and falling to initial level 120 to 180 minutes after glucose administration.

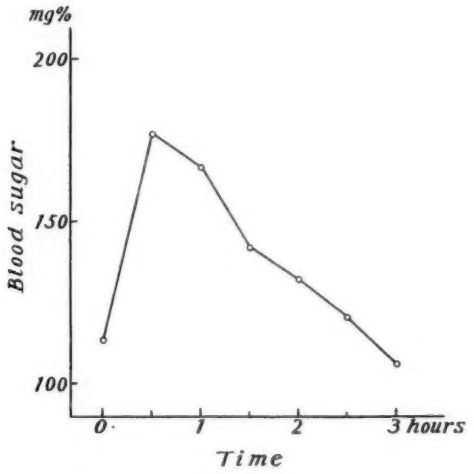

Fig. 1. Glucose tolerance test of normal rats.

Comparison of glucose tolerance in each group with this normal curve was as follows:

(a) The standard diet group. Blood sugar level in glucose tolerance test remained in the normal range. The peak level was not elevated higher than 200 mg% with negative urine sugar (Figure 2).

(b) The high-protein diet group. Initial level was not different from the normal one, some showing higher level in peak than 200 mg% and the time falling to the initial level being retarded in both high- and isocaloric groups. Decreased glucose tolerance was observed in one rat in this high-caloric group, the maximum blood sugar level reached 254 mg% and positive urine sugar was found in glucose tolerance test (Figure 3).

(c) The high-fat diet group. The tendency of high initial level, higher maximum and prolonged recovery to intial level was more remarka-

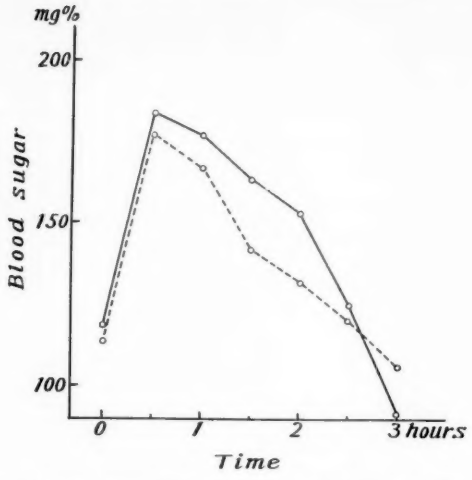

Fig. 2. Glucose tolerance test of a rat in the group of standard diet. The broken lines in Figures 2 to 5 represent the glucose tolerance curves of normal rats.

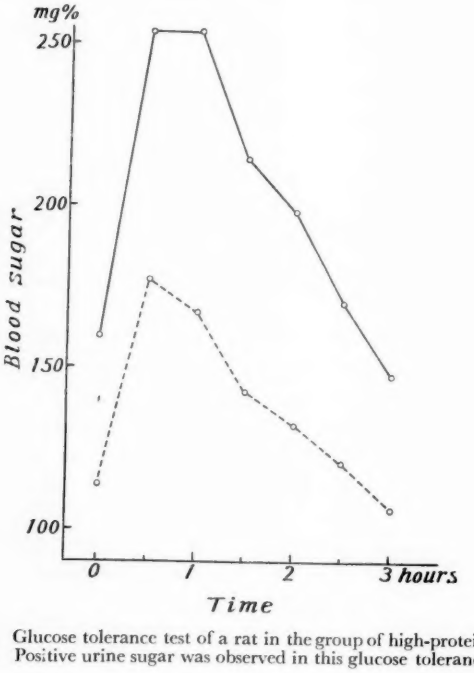

Fig. 3. Glucose tolerance test of a rat in the group of high-protein high caloric diet. Positive urine sugar was observed in this glucose tolerance test.

ble than normal glucose tolerance curve. But positive urine sugar was not found in glucose tolerance test (Figure 4).

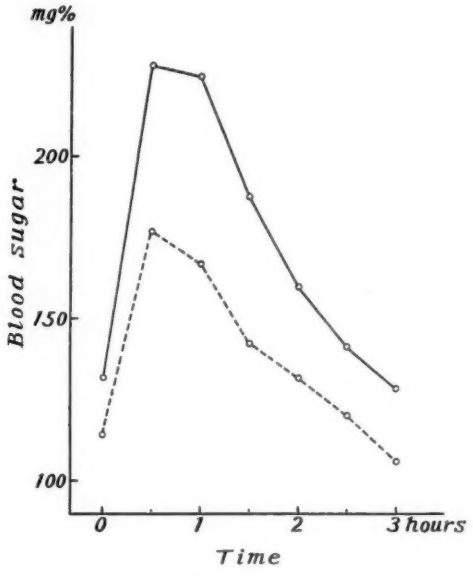

Fig. 4. Glucose tolerance test of a rat in the group of high-fat high-caloric diet.

(d) The high-protein high-fat diet group. Markedly decreased glucose tolerance was perceived in this group comparing with above groups. Positive urine sugar was observed in three rats of this high-caloric group in which the maximum blood sugar level reached 330 mg% (Figure 5).

Glucose tolerance tests of all groups were summarized in Figure 6 and Table II, in which blood sugar levels are the mean values in ten rats in each group. Markedly decreased glucose tolerance was observed in the group of high-protein high-fat high-caloric diet, followed by the group of high-fat high-caloric diet. Glucose tolerances were moderately decreased in other dietary groups compared with standard dietary group.

Glucose uptake rate of diaphragm

Glucose uptake rate is summarized in Figure 7, in which the rate is expressed as proportion to that of the standard dietary group. It was found to be reduced in the groups of high-protein and high-protein high-fat diet, and particularly extremely in the group of high-fat diet.

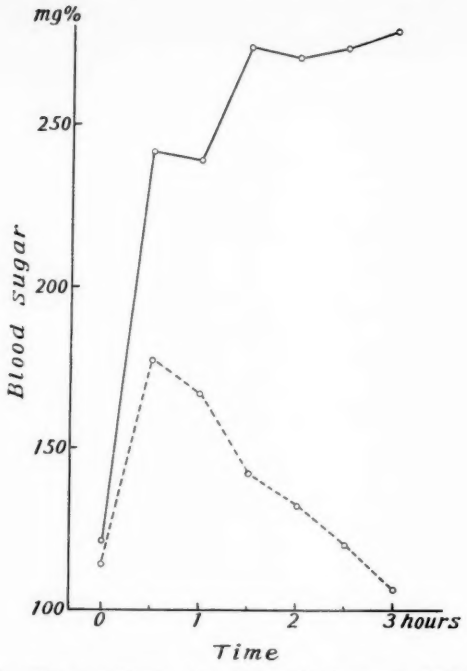

Fig. 5. Glucose tolerance test of a rat in the group of high-protein high-fat high-caloric diet. Positive urine sugar was observed.

Blood acetone level

The free acetone content in blood was found elevated in all groups except for the standard diet group (Figure 8).

Pathohistological changes

The pathohistological changes observed in these dietary groups were most prominent in the group of high-protein high-fat high-caloric diet, as compared with that in the standard dietary group, in the other groups showing similar changes in degree to those of the group of standard diet.

(a) Langerhans' islets of pancreas. The islets were enlarged and the β-cells were swollen and degranulated in the group of high-protein high-fat diet (Figure 9).

(b) Adrenal glands. Thickening of fascicular layer was found in the group of high-protein high-fat diet compared with the standard dietary group.

(c) Anterior pituitary lobe. There were found no remarkable changes in this organ in all dietary groups except for in the group of high-protein high-fat diet, in which small basophilic cells were found to be

TABLE II

Average Glucose Tolerance Test Two Months after Feeding with Different Diets

	Blood sugar level and standard deviation (mg%)						
	before glucose loading	Time (hrs) after glucose loading					
		0.5	1.0	1.5	2.0	2.5	3.0
Standard	125 ±15.7	167 ±16.4	164 ±18.8	147 ±21.0	135 ±11.8	121 ±10.2	113 ±22.2
High-protein (Isocal.)	126 ±14.8	185 ±33.5	179 ±29.2	162 ±29.5	145 ±23.1	139 ±23.0	133 ±13.5
High-protein (High-cal.)	125 ±23.9	184 ±19.7	175 ±38.4	150 ±30.3	140 ±23.0	125 ±23.0	120 ±15.3
High-fat (Isocal.)	112 ±14.2	190 ±28.1	185 ±23.0	161 ±17.5	138 ±25.7	136 ±23.2	133 ±23.8
High-fat (High-cal.)	140 ±10.6	188 ±25.3	200 ±31.8	187 ±30.9	169 ±29.3	149 ±25.2	136 ±15.1
High-protein high-fat (Isocal.)	115 ±12.8	180 ±19.4	175 ±19.2	159 ±19.5	150 ±17.3	145 ±23.3	140 ±19.6
High-protein high-fat (High-cal.)	136 ±13.2	190 ±23.2	213 ±23.3	191 ±42.9	178 ±43.5	170 ±31.4	163 ±33.2

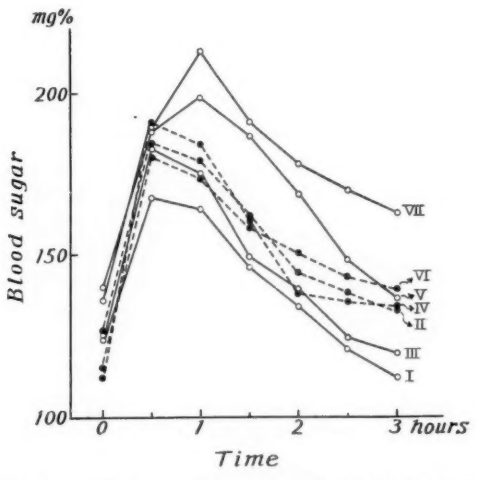

Fig. 6. Glucose tolerance curves of the animals fed with seven different diets. The blood sugar levels are the mean values of ten rats in each dietary group. I.. the group of standard diet. II.. the group of high-protein isocaloric diet. III.. the group of high-protein high-caloric diet. IV.. the group of high-fat isocaloric diet. V.. the group of high-fat high-caloric diet. VI.. the group of high-protein high-fat isocaloric diet. VII.. the group of high-protein high-fat high-caloric diet.

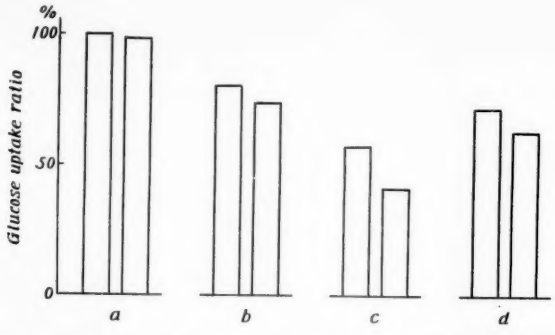

Fig. 7. Glucose uptake rate of diaphragm. Each value is expressed as proportion to that of standard dietary group. a. standard dietary group. b. the group of high-protein high-caloric diet. c. the group of high-fat high-caloric diet. d. the group of high-protein high-fat high-caloric diet.

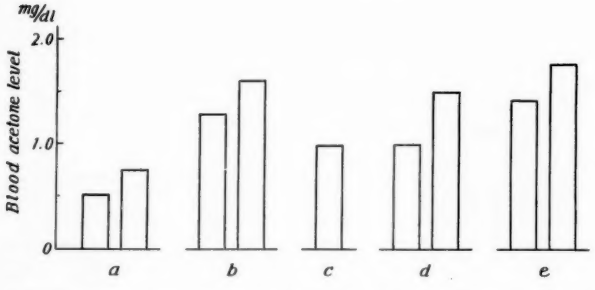

Fig. 8. Blood free acetone. a. the group of standard diet. b. the group of high-protein isocaloric diet. c. the group of high-fat isocaloric diet. d. the group of high-protein high-fat isocaloric diet. e. the group of high-protein high-fat high-caloric diet.

increased in number.

Discussion

It was Tejning[15] who studied systematically on the dietary effect upon the carbohydrate metabolism, although numerous studies have been reported on the role of diet in the occurrence of experimental diabetes mellitus.

He observed the difference between the size of Langerhans' islets in the animals fed with different diets. The largest islets were found in the group of standard diet and the smallest in the group of high-fat diet.

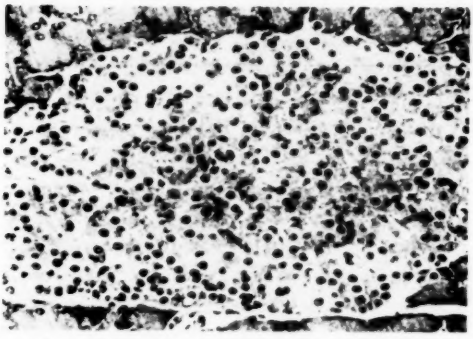

Fig. 9. Enlarged Langerhans' islet. β-cells are swollen and degranulated. In the group of high-protein high-fat high-caloric diet. Gomori's chrome alum hematoxylin stain.

By Houssay and Martinez[7], lard was also diabetogenic in the partially pancreatectomized animals among the numerous diets.

Seven dietary systems were used to survey the dietary influence upon the carbohydrate metabolism in our experiment. The composition of standard diet used in our experiment is the widely used one in rats, which is unexpectedly similar with therapeutic formula for diabetic patients in our clinic[16]. On the other hand, the amount of fat and protein in the high-protein high-fat high-caloric diet coresponds to one which is supposed as large amount in human diet[17].

It is shown in our experiment that high-protein high-fat can decrease the carbohydrate tolerance for two months.

Kotake and coworkers[9] showed that diabetes mellitus was induced in the animals fed with high-protein high-fat diet for about 170 days. However, it is obvious from our experiment that such diet decreased carbohydrate tolerance, or latent diabetic state was induced in the rats fed with such diet according to the results of glucose tolerance tests, glucose uptake ratio of diaphragm and free acetone level of blood. These latent diabetic states could not be found by the estimation of fasting blood sugar, but could be perceived by glucose tolerance test, which, we suppose, is the best method in the experiment to discover latent diabetes mellitus.

How is the decreased carbohydrate tolerance induced in rats fed with high-protein high-fat high-caloric diet?

In our experiment, diabetes mellitus or decreased carbohydrate tolerance could not be induced solely by protein or fat. Protein is supposed to be rather protective against occurrence of diabetes by alloxan according to the results of Houssay and Martinez[7], and fat is illustrated as diabetogenic factor by many authors.

Then carbohydrate tolerance of the animal might be decreased only when the animal is fed with high-protein high-fat diet. Kotake[9] attributed this mechanism to xanthurenic acid derived from tryptophan in pyridoxin deficiency. However, because carbohydrate metabolism was more disordered in this high-caloric than isocaloric diet, high-caloric diet might have more significance in the occurrence of decreased carbohydrate tolerance.

Secondry, it is suggested from histological studies that β-cells in Langerhans' islets are overstrained and exausted by such high-caloric diet and decreased carbohydrate tolerance is induced as the result. This overstrain of Langerhans' islets might be concerned with obesity because gain in body weight is remarkable in the group of high-protein high-fat diet.

Thirdly, pituitary adrenal system is presumed to play a part in progress of decreased carbohydrate tolerance, which conformed an evidence in histological studies on pituitary and adrenal glands[19].

Summary

One hundred and five normal rats were fed with seven different diets, among which carbohydrate tolerance was found to be decreased extremely in the group fed with high-protein high-fat diet for two months.

Three factors, such as xanthurenic acid, overstrain of Langerhans' islets and pituitary adrenal system were discussed to be presumably responsible for this mechanism.

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Diet and Experimental Diabetes

II. The Dietary Influence on the Occurrence of Alloxan Diabetes in Rats

By

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Previously we reported that the carbohydrate tolerance of normal rats was decreased in the group of high-protein high-fat especially high-caloric diet[1]. Then it was suggested that this disorder of carbohydrate metabolism is due to the lowered utilization of glucose in the peripheral muscle, which is concerned with the function of Langerhans' islets and pituitary and adrenal system from the pathohistological studies.

If the function of Langerhans' islets will be lowered by high-protein high-fat diet, experimental diabetes will be easily induced in the animals fed with this diet by the administration of less amount of alloxan than the amount commonly used.

Then we successively studied the dietary influence upon the occurrence of diabetes mellitus induced by alloxan administration in rats.

Experimental

Materials and Method

Animals used in this experiment were those rats which were fed with seven different diets for two months[1].

Five % aqueous solution of alloxan was injected to the femoral veins of each five animals of all dietary groups in amount of 40 mg. per kg. body weight and of other five animals in amount of 30 mg. per kg. body weight, respectively, two months after feeding.

Fasting blood sugar of all animals was measured every one or two days for about two weeks after alloxan administration.

Animals survived were subjected to glucose tolerance test two weeks after the administration of alloxan.

Glucose tolerance test and estimation of blood sugar level were performed by the same methods as previously reported[1][2].

Results

Occurrence of diabetes mellitus by alloxan administration

Diabetes mellitus was induced in almost all the animals of every group by the administration of 40 mg. per kg. body weight of alloxan. By the administration of 30 mg. per kg. body weight of alloxan, however, the occurrence rate of diabetes mellitus was 100% in the group of high-protein high-fat diet, while it fell to 40% in the group of standard diet and nearly midways of these percentage in the other dietary groups. This is summarized in Table I.

TABLE I

Occurrence of Diabetes Mellitus after Alloxan Administration

	Diet group													
	Standard		High-protein (Isocal.)		High-protein (High-cal.)		High-fat (Isocal.)		High-fat (High-cal.)		High-protein high-fat (Isocal.)		High-protein high-fat (High-cal.)	
Dosis of alloxan (mg/kg)	40	30	40	30	40	30	40	30	40	30	40	30	40	30
No. of rat	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Ditto diabetic within 3 days	5	2	5	2	4	3	4	0	4	3	5	2	5	5
Ditto not diabetic	0	3	0	3	1	2	1	5	1	2	0	3	0	0
Ditto dead within 7 days	3	1	3	0	1	1	0	0	3	1	1	0	1	0
Ditto surviving	2	4	2	5	4	4	5	5	2	4	4	5	4	5

Progress of diabetes mellitus after alloxan administration

Death ratio within seven days after alloxan administration is high in the groups of standard diet, high-protein isocaloric diet and high-fat high-caloric diet with the administration of 40 mg. per kg. body weight of alloxan, being low in the animals of other groups (shown in Table I).

The changes in blood sugar level after alloxan administration were able to be classified as follows;

(a) persistent diabetization: hyperglycemia and glycosuria were observed during whole period,

(b) recovery after diabetization: transient hyperglycemia and glycosuria were observed, and

(c) non-diabetization: neither hyperglycemia nor glycosuria developed.

These are shown in Figure 1. The rate of persistent diabetization was found high in all the groups with the administration of 40 mg. per kg. body weight of alloxan, while the rate of occurrence was the highest

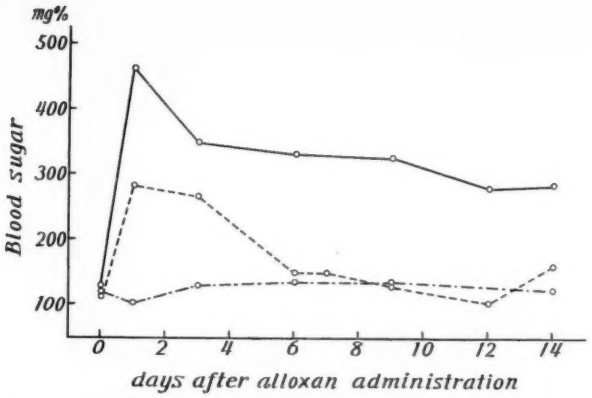

Fig. 1. The changes in fasting blood sugar after alloxan administration. The solid, broken and chain lines represent the fasting blood sugar levels of the animals persistently diabetized, recovered after diabetization and non-diabetized, respectively.

TABLE II

Progress of Diabetes Mellitus after Alloxan Administration

	Diet group													
	Standard		High-protein (Isocal.)		High-protein (High-cal.)		High-fat (Isocal.)		High-fat (High-cal.)		High-protein high-fat (Isocal.)		High-protein high-fat (High-cal.)	
Dosis of alloxan (mg/kg)	40	30	40	30	40	30	40	30	40	30	40	30	40	30
No. of rats persistently diabetized	5	1	4	2	4	1	1	0	4	1	2	0	3	3
Ditto recovered after diabetization	0	2	1	0	0	2	3	0	0	2	3	2	2	2
Ditto not diabetized	0	2	0	3	1	2	1	5	1	2	0	3	0	0

and none of non-diabetization was detected in the group of high-protein high-fat high-caloric diet among the groups administered with 30 mg. per kg. body weight of alloxan.

The data are summarized in Table II.

Glucose tolerance tests two weeks after alloxan administration

Blood sugar level was higher than 250 mg. % before glucose administration, higher than 360 mg. % in its peak in all animals with course of persistent diabetization (Figure 2).

Glucose tolerance was found to be decreased even in the animals

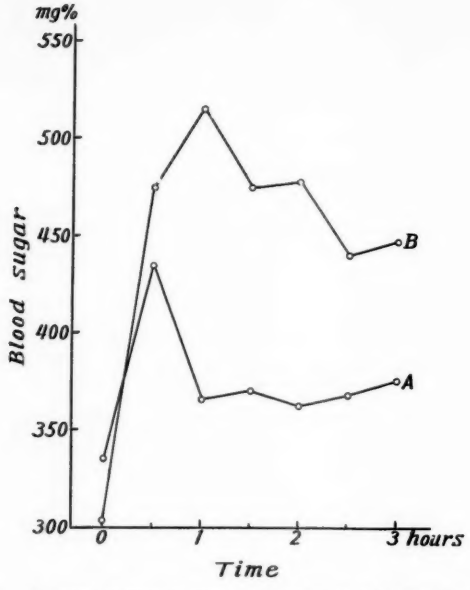

Fig. 2. Glucose tolerance test of persistently diabetized animals. A. glucose tolerance curve of the animal fed with standard diet. B. glucose tolerance curve of the animal fed with high-fat high-caloric diet.

with apparently cured diabetes and more markedly decreased in other groups than in the standard dietary group (Figure 3).

It is to be noted that decreased glucose tolerance was also observed in apparently non-diabetized animals (Figure 4).

Furthermore, these decreased glucose tolerances were observed more remarkable in the group of high-protein high-fat diet or other groups as compared with the standard dietary group.

Discussion

The dosis of alloxan inducing diabetes mellitus by the intravenous administration has been reported as 40 to 50 mg. per kg. body weight in rats[2] and this was proved in our experiment. Diabetes mellitus, however, could also be induced by the administration of 30 mg. per kg. body weight of alloxan which was been considered as non-diabetic dosis, especially in 100% in the group of high-protein high-fat high-caloric diet.

It is suggested that high-protein high-fat high-caloric diet intensifies the occurrence of diabetes mellitus induced by the administration of alloxan and can spare the amount of alloxan to induce diabetes mellitus,

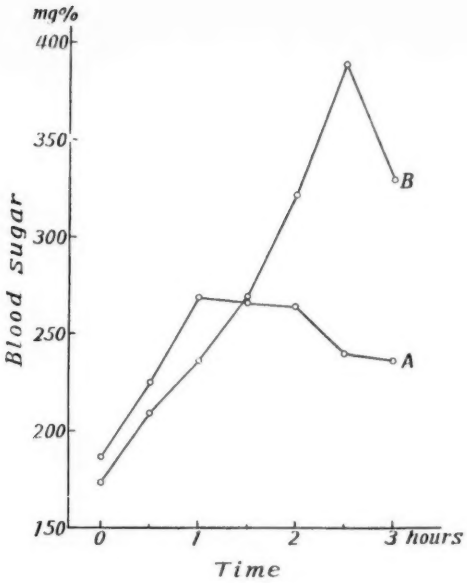

Fig. 3. Glucose tolerance test of the animals recovered after diabetization. A. glucose tolerance curve of the animal fed with standard diet. B. glucose tolerance curve of the animal fed with high-protein high-fat high-caloric diet.

because this diet formula decrease the glucose tolerance of normal rats by itself, as we previously reported[1].

Although Houssay and Martinez[4] reported that diabetes mellitus occurred thoroughly in the animals subtotally pancreatectomized and fed with high-lard diet, compared with other diets, the alloxan sparing effect of this high-fat diet was not observed in our experiment.

Death ratio after alloxan administration was high in high-lard dietary group according Houssay and Martinez's experiment, whereas such result was not revealed in our experiment. This difference might be explained by the fact that alloxan was injected intraperitoneally in the dosis of 160 mg. per kg. body weight in their experiment, while intravenously 30 to 40 mg. per kg. in ours.

We tried to classify the progress of alloxan diabetes into three groups. Hitherto, these transient or non-diabetized animals have been neglected in the experimental studies of alloxan diabetes. But it should be emphasized that impairment of carbohydrate metabolism can be found by the glucose tolerance test even in the animals, which seem to be recovered or non-diabetized by the routine examinations such as the estimation of

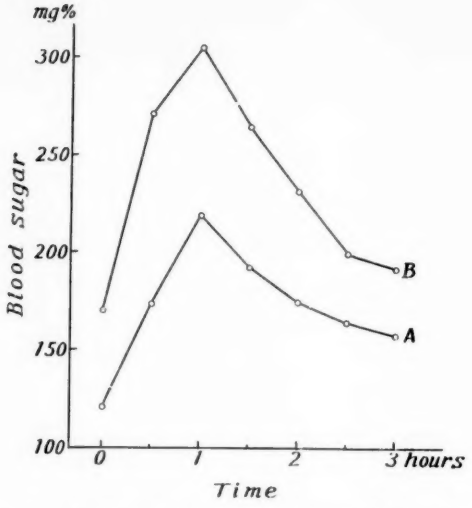

Fig. 4. Glucose tolerance test of the non-diabetized animals. A. glucose tolerance test of the animal in the group of standard diet. B. glucose tolerance test of the animal in the group of high-protein high-fat isocaloric diet.

fasting blood sugar and urine sugar, and that the estimation of fasting blood sugar and urine sugar is not enough to observe the progress of alloxan diabetes whereas glucose tolerance test should be carried out at least.

Conclusively, it is suggested from our experiment that the high-protein diet with high-fat is harmful and that the standard diet, well balanced in three food stuffs, is superior to other diet formulas both prophylactically[1] and therapeutically[2] in experimental diabetes mellitus in rats.

Summary

Although almost animals were induced by the administration of 40 mg. per kg. body weight of alloxan, occurrence of diabetes mellitus by 30 mg. per kg. body weight of alloxan is the highest in the group of high-protein high-fat high-caloric diet among various dietary groups.

Progress of diabetes by alloxan administration was classified into three groups, namely persistent diabetization, recovery after diabetization and non-diabetization.

Furthermore it was emphasized that glucose tolerance was revealed to be decreased equally in the groups of recovery after diabetization and non-diabetization.

It was suggested that high-protein high-fat diet intensified the induc-

tion of diabetes mellitus with the administration of small amount of alloxan and, therefore, could spare the amount of alloxan administered to induce experimental diabetes.

References

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